Are anthropogenic fibres a real problem for red mullet (*Mullus barbatus)* from the NW Mediterranean?

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ABSTRACT

Microfibres are among the most prevalent type of microplastics in marine environments. Man-made fibres derived from cellulose are also worldwide distributed, but often confused with synthetic plastic fibres and consequently neglected. All these fibres may affect adversely aquatic organisms but their levels and potential effects in wild fish remain unknown. *Mullus barbatus* were analysed to study anthropogenic fibre (AFs) ingestion, at temporal and geographical scale, and to assess their possible effects in fish health condition. AFs were present in 50% of fish digestive tract 1.48 AF / individual (SD = 1.98). An increase of 46% in AF ingestion have been observed in 2018 compared to 2007 in Barcelona. AF ingestion also increase in 20% when compared Barcelona to a less human impacted area (Blanes). Visual characterisation of fibres by typologies, corroborated by Raman spectroscopy, allowed classification and identification of 88% of AFs as cellulosic (57 %) and synthetic polymers (PET) (31%). The only histopathological alterations were cysts of unknown etiology, and the most abundant parasites were nematodes in all sampling stations. None of these alterations, parasites or other fish health indicators (condition indices) reflect an effect of AFs ingestion.

1. Introduction

Pollution by organic synthetic polymers, commonly known as plastic, in the ocean was first reported by scientists in the 1970s (Carpenter et al., 1972; Carpenter and Smith, 1972; Colton et al., 1972) and it has drawn tremendous attention in recent years from scientists to media and society (Law, 2017). Currently, plastic pollution is a major threat to marine and terrestrial ecosystems globally (Derraik, 2002). Worldwide, it is estimated that between 4 and 12 million tonnes of plastic enters the world's oceans annually, mainly from coastal inputs (Jambeck et al., 2015) and it has become ubiquitous in the ocean in only few decades. Small plastic debris, classified as microplastics (MPs) if their size is smaller than 5 mm (Hartmann et al., 2019) are also found globally (Cózar et al., 2014) floating or deposited in the seafloor and in the most remote locations including the deep seafloor (Woodall et al., 2014), uninhabited islands (Lavers and Bond, 2017), the Arctic (Cózar et al., 2017) and coastlines and the surrounding waters of the Antarctica (Waller et al., 2017), and particularly in coastal shallow populated areas (Alomar et al., 2016). MPs can be also classified attending their shape criterion which includes beads, fibres, films and fragments (Hartmann et al., 2019). Fibres are among the most prevalent type of MPs observed in the marine environment (Browne et al., 2011), so fibre pollution has gained much attention in last years. Anthropogenic fibres (AFs) (Lahens et al., 2018) refer not only to the plastic / synthetic fibres from petrochemical origin (i.e. polyester, polyamide, polypropylene, etc.), but also to non-synthetic fibres, which include artificial fibres from artificial cellulose or silk (i.e. viscose, rayon) and the natural fibres (i.e. cotton, wool), all of them used in textile and apparel industries. Non-synthetic fibres coming from the textile industry or urban wastewater treatment plants also reach aquatic environment. These non-synthetic fibres, despite being inherently unnatural, have received little environmental attention (Stanton et al., 2019), unlike plastic ones. They are also worldwide distributed (Gago et al., 2018), even in the gastrointestinal tract of organisms, and can be often confused with synthetic plastic fibres due to their similar morphological features (Remy et al., 2015; Savoca et al., 2019). All these anthropogenic fibres, with their additives or dyes and their capability to absorb other contaminants to their surfaces, have been suggested affecting adversely in aquatic organisms (Burgos-Aceves et al., 2018a,

2018b; Faggio et al., 2018; Prokić et al., 2019). However, the levels of this pollution and its potential effects in wild fish remain unknown.

The Mediterranean Sea is a semi-enclosed, highly populated basin, exposed to heavy coastal pressures, such as, maritime traffic, waste discharges and river inputs which determine densities of marine debris including marine plastics (Alomar et al., 2017; Barnes et al., 2009; Deudero and Alomar, 2015; Jambeck et al., 2015). It is estimated that between 1000 and 3000 tons of plastic are floating on the Mediterranean Sea (Cózar et al., 2015). In the NW Mediterranean, AF's, have been reported in several seafloor environments such as estuarine, coastal areas (Alomar et al., 2016; Simon-Sánchez et al., 2019) and deep sea (Sanchez-Vidal et al., 2018). Their ingestion by marine organisms has also been reported for both offshore and inshore fish (Bellas et al., 2016), as well as for deep-sea organisms such as fish and crustaceans (Carreras-Colom et al., 2018; Romeu et al., 2016). *Mullus barbatus* Linnaeus, 1758, commonly named red mullet, is a benthic fish species widely spread in the Mediterranean Sea and the North Eastern Atlantic which inhabits the continental shelf in gravel, sandy and muddy bottoms up to 500 m depth (Lloris, 2015). Due to its diet and feeding behaviour (Bautista-Vega et al., 2008), this species is in constant contact with sediment, and therefore, it is also exposed to the pollutants, contained in it (Van Cauwenberghe et al., 2015). Thus, it has been widely proposed as a sentinel species for a number of pollutants (Bray et al., 2019; Carreras-Aubets et al., 2012). AFs ingestion by red mullet has been reported in several areas of the Mediterranean Sea such as Turkish shore, Adriatic, Ionian and Tyrrhenian Seas and Mediterranean Spanish Coast (Avio et al., 2015; Bellas et al., 2016; Capillo et al., 2020; Digka et al., 2018; Güven et al., 2017). Although these studies have shown red mullet ingesting AFs, their possible effects on fish have not been addressed, which is an important issue for a reliable risk assessment of these pollutants.

Negative effects associated to AFs and MPs ingestion have been observed under laboratory experiments exposed to distinct serials of concentrations of pollutants, though these ranges are acute and high environmentally-unrealistic concentrations (Cunningham and Sigwart, 2019). Health status of wild fish populations are generally addressed by integrating many indicators, from fish condition to cellular or tissue alteration and parasite infestation, which allow obtaining an overview of population wellbeing. Fish condition indices can alert of the occurrence of diseases or other physiological features before mortality events. For instance, hepatosomatic and

gonadosomatic index, which are widely used both in ecological studies and evaluation of fisheries of wild fish stocks (Brosset et al., 2015; Stevenson and Woods, 2006), give information about the physiological status related to the capacity / accumulation of short-term reserves and the reproductive capacity, respectively. Fulton's condition factor is the main indicator of fattening of individual / population (Nash et al., 2006) and fasting or feeding intensity can be obtained by the stomach fullness index (Hyslop, 1980). In the NW Mediterranean few studies have suggested oxidative stress and effects in fish condition related to ingested AFs in wild fish (Alomar et al., 2017; Compa et al., 2018). Moreover, sub-lethal environmental stress, e.g. produced by pollutants, can be reflected in cellular or tissue alterations of different organs of fish. The presence or changes in the intensity of these histopathological alterations caused by pollutants such as heavy metals o organic compounds, can be assessed by the microscopic observation of target sensible organs (Au, 2004; Costa, 2018; Stentiford et al., 2003). The most target organs used in histopathology to assess the effects caused by aquatic pollutants are liver and gills, but also digestive tract, kidney and gonads are highly relevant (Costa, 2018). Histopathological alterations caused by plastics (eg. Inflammation, cell death, necrosis) are also described in fish after MP exposure in laboratory conditions (Ahrendt et al., 2020; Kögel et al., 2020), but studies in wild marine fish are really scarce. Finally, fish parasit fauna is a widely useful health indicator of both organisms and ecosystems, since parasite populations can either increase or decrease against environmental changes depending on their life cycle and the nature of pollutant (Mackenzie et al., 1995; Marcogliese, 2005; Sures, 2001). However, studies linking the presence of plastics with parasites are extremely scarce. Hernandez-Milian et al. (2019) hypothesized that parasite aggregations within intestines could retain microplastics and cause their aggregation. Therefore, besides being bioindicators of pollutants of marine ecosystems, parasites could increase the accumulation of microplastics within biota and therefore increasing the risk of damage to their health.

In the present study, we analysed the prevalence, size and composition of anthropogenic fibres in the digestive tract of red mullet individuals comparing two different years with a 10-year gap (2007 and 2018) and different locations along the Spanish NW Mediterranean Sea. The aims of the present study are: 1) To assess the presence of AF in the digestive tract of *M. barbatus* from the NW Mediterranean Sea, and to infer: 2) differences between the prevalence and typologies

of current ingested AF and those ingested 10 years ago; 3) geographical variations of the prevalence and typologies of the ingested AFs between two localities (high and less impacted area); 4) to assess the potential effect of AFs on the fish health by applying health indicators such as biological and condition index, tissue histological alterations and the analysis of parasitofauna; and finally 5) to discuss the possible source or origin of the different kind of AFs within *M. barbatus*.

2. Materials and Methods

2.1. Study area and sample collection

A total of 118 *Mullus barbatus* were captured at depths between 60 and 130 m from the continental shelf off the Catalan coast (NW Mediterranean) within the framework of BIOMARE (Spanish Ministry of Science and Innovation) and SOMPESCA (Department of Agriculture, Livestock, Fisheries and Food, Catalonia, Spain) multidisciplinary projects (Table1). Two areas were sampled, about 5 miles off Barcelona and northern nearby Blanes offshore (Fig.1). Barcelona city and the near metropolitan zone is an industrialized and densely populated coastal area compared to Blanes. While Barcelona shore is under the influence of two main rivers (Llobregat and Besòs) which, in addition to typical seasonal abrupt discharges have a continuous flow regime throughout all year, the shore off Blanes is under the influence of a single smaller river (Tordera river) that has mainly a seasonal pattern of water discharges. Fish were collected aboard commercial and scientific fishing vessels during 2007 (48 specimens) from Barcelona and with commercial fishing vessels during 2018 (70 specimens) at two different sites (same location off Barcelona as 2007 and Blanes), in both cases at two seasons (spring and summer) (Table1). Two different fishing gears were used: a semi-balloon otter trawl, OTSB14 (Merrett and Marshall, 1980) and a commercial fishing trawl (BOU). Fish were immediately fixed in 10% buffered formalin and transported to the laboratory for analyses. An abdominal incision was made in order to improve the fixation process of internal organs.

2.2. Laboratory procedures

Once at the laboratory, each specimen was measured to the nearest mm (total length = TL and standard length = SL), weighed to the nearest g (total weight = TW) and dissected. To minimize airborne contamination all procedures were made in a laminar flow cabinet, which was previously

cleaned, as well as the laboratory equipment and tools rinsed with deionized (50 µm) water. Nitrile gloves and exclusive cotton lab coats were also used all time. The gastrointestinal tract was removed by dissection following previous procedures (Lusher et al., 2013), from the top of the esophagus to the anus. Stomach (SW), liver (LW) and gonads (GW) were weighed inside the laminar flow hood using a precision scale to the nearest mg. The spleen was also removed and then the individuals were weighed again to the nearest g (eviscerated weight = EW). All organs including the dissected gastrointestinal tract (stomach, caeca and intestines) were stored separately in filtered 70% ethanol in individual glass vials previously rinsed with deionized filtered (50 µm) water for subsequent observations.

2.3. Anthropogenic items (fibres and fragments) isolation and visual inspection

The content of the stomach, caeca and intestine was carefully screened under a stereoscopic binocular at x5 to x45 magnification. To prevent background contamination, the stereomicroscope and work area was isolated from the exterior by an isolation device adapted from the one proposed by Torre et al. (2016) and the interior was carefully washed before its use to minimize the presence of airborne anthropogenic items. The laboratory dissection material was also rinsed with filtered deionized water twice before its use. Procedural controls, which consists in uncovered Petri dishes filled with filtered water, were placed inside and outside of the isolation device during digestive content screening in order to assess potential airborne contamination. Only fibre shaped items were found in both controls. Contamination found in the inside controls (average values of 0.22 fibres per digestive sample screened) was 3.6 times less abundant than contamination in outside controls, thus pointing out the efficiency of the isolation device in reducing potential contamination. Fibres found in inside controls were clean and always appeared on the surface of the water (pointing out that they were deposited from air). Therefore, fibres from digestive contents were only counted if they were clearly embedded in the digestive content and/or with detritus attached and were clearly differentiated from those floating on the surface excluded hereafter. Because of that, no correction factor was applied to the final values of fibres reported.

To avoid misidentification of AFs with vegetal remains (e.g. seagrass or algae) a selection criterion adapted from proposed by Hidalgo-Ruz et al (2012) were used for fibres. AFs detected were collected and mounted between glass slides in filtrated deionized water and observed under the microscope. When they presented vegetal morphological features such as cellular or organic structures, were discarded. Length and mean cross section (based on three random measures) were obtained for fibres, and only cross-section for fragments. Images were obtained by a Leica camera model: CTR 5000 attached to the Leica microscope model: DM 5000 DB and measured by an image-processing piece of software (ProgRes® C3).

Anthropogenic items were counted for each individual and their localization within the digestive tract (stomach, caeca and intestines) was annotated.

The AFs found in the digestive tract of fish were carefully observed under the microscope, characterized and classified into distinct typologies according to their morphological features: general appearance (GA) and microscopic appearance (MA) (cross-section shape, patterns of the fibre's body, shape and appearance of the ends, breakages and alterations of the fibre's body, birefringence and colour) (Robertson et al., 2017). Prevalence of each typology was calculated as percentage of each fibre type respect to the total amount of AFs.

2.4 Raman characterization of AFs

After visual classification, 25% of these fibres of each type (39 AFs in total) were randomly selected for their identification by Raman scattering.

Raman spectra were measured using a WITec Alpha300RA piece of equipment. The experiments were performed under ambient conditions and employing low acquisition times (typically 100 ms) and moderate laser powers (488nm excitation, typically 1.5 mW except for cotton-like fibres, for which it was increased) to minimize laser induced degradation of the fibres. Fibres were imaged (several hundreds of spectra per image) through a 40x objective and using a motorized stage. Data were clustered using the Witec Project 5 software to account for in/out of the fibre positions. All spectra within each fibre were then averaged after removing the cosmic rays hitted pixels and background. The measured spectra were compared against a custom library, with target known polymers (see supplementary material), and commercial Raman library BioRad KnowItAll® Informatics System – Raman ID Expert (2015) software. Hit Quality Index (HQI) was associated

with each reference spectrum in order to polymer identification. HQI is a numerical measure of the closeness of fit between the unknown spectrum and each reference spectrum. The minimum match value between the obtained spectra and the library used for characterization was 70%.

2.5. Health assessment

Fish condition was assessed by the gonadosomatic index (GSI = (GW/TW) x 100), the hepatosomatic index (HSI = (HW/TW) x 100) and the Fulton's body condition factor (K = EW x $100/(SL)^3$). Feeding intensity was measured by the stomach fullness index (FULL = (CW/EW) x 100), which was calculated using the total stomach content weight (CW).

A portion of gonad, liver, spleen, kidney, stomach (after anthropogenic items isolation) and gills were embedded into paraffin and processed by routine histology. A section (5 µm) of each organ was stained with Haematoxylin and Eosin for histopathological assessment. All histological samples were completely screened in order to detect histological alterations under the microscope. The aim of this analysis was to detect the possible histological alterations (e.g. inflammation, death cells; see Kögel et al. 2020) which may be related to the ingestion of AFs or associated toxic substances, but not to detect the fibres themselves. The spleen was chosen for the melanomacrophagic centres (MMC) quantitative study due to the easiness of ablation of this organ and the possibility to obtain complete radial sections (Fournie et al., 2001; Manera et al., 2000). For these purpose, three fields of view (0.23 mm^2/screen) were randomly selected from each section of spleen at 200x and examined microscopically. Area and number of MMC (Mean area = MA.MMC and number = nMMC) of each field were measured using a MicroComp Integrated Image Analysis System and a size discriminator was used to eliminate objects smaller than 100 µm2.

External surfaces and gills were checked macroscopically for ectoparasites and the rest of organs, including stomach, caeca, intestine (after AFs screening) and the internal body wash were carefully inspected for endoparasites under stereomicroscope. Digeneans and cestodes were stained with iron acetocarmine and permanently mounted in Canada balsam. Nematodes were temporally cleared and mounted in glycerin before identification. Parasite were identified under optic microscope to the lowest possible taxonomic level (see supplementary material).

2.6. Data analysis

The prevalence of AF was calculated as the proportion of fish containing AF within their digestive tract with respect to the total number of fish. The number of AFs were determined for each individual (nAF) .Total length of AFs (TLAF) was calculated as the addition of the length of all AFs observed inside the digestive tract of each individual in order to infer the total amount of AFs inside each part of the digestive tract. Data were tested for normality using the Shapiro-Wilk test in order to assess differences in the number (nAF) and size (TLAF) found in different parts of fish digestive tract. When data did not satisfy the assumptions of normality non-parametric Kruskal-Wallis were used.

In order to characterize the fibre size, each AF of the whole digestive were classified by size into four clusters by partitioning around medoids (PAM) clustering (Kaufman and Rousseeuw, 1990), by the PAMK function implemented in the package fpc 2.2-3 in R Studio 3.5.0. Chi-Squared test was used for testing changes in proportions of AFs (AF fish prevalence, size categories and typology) or alternatively, Fisher exact test was used when small samples and expected values were < 5.

As preliminary data analyses did not show significant differences in AFs (in neither number nor length) between spring and summer samples within the same year, neither in 2007 nor 2018 (data not shown), seasonality was not considered as a factor in the forthcoming analyses. The analyses between 2007 and 2018 was conducted using the subset of samples from Barcelona (2007-2018), and the geographical comparison was performed with the subset of samples from 2018 (Barcelona-Blanes).

To assess differences in prevalence of AFs, nAF and TLAF by year (2007 and 2018) and locality (Barcelona and Blanes) generalized Linear Models (GZM) (binary logistic model and negative binomial model) and general linear model (GLM), respectively were used, with SL as a covariate.

Prevalence of the histological alterations (Cysts of Unknown etiology) was calculated as the proportion of fish containing the alteration with respect to the total number of fish. Parasite prevalence (P%) and mean abundance (MA) were calculated following previous studies (Bush et al., 1997), and parasite richness (R) and parasite diversity for each individual was also calculated, using the Shannon Index (H').

Differences in biological indices were tested also by year and locality, with SL as a covariate: K and HSI using GLM; GSI, FULL, splenic melanomacrophagic centres (nMMC and MA.MMC) and parasites (total number of parasites = nPAR) using GZM (gamma with log link and negative binomial model); and prevalence of histological alterations by GZM (binary logistic model).

To test possible correlations between anthropogenic fibres (nAF and TLAF) with fish histological alterations and parasite prevalence (for each taxonomic group) GZM (binary logistic model) were also used. To test correlations between anthropogenic fibres (nAF and TLAF) with parasites descriptors (R and H') GZM (negative binomial model) and GLM, respectively, were used. In order to test the potential implication of abundance of parasites (total abundance and for each taxonomic group) within digestive tract in the retention of anthropogenic fibres (nAF and TLAF), GZM (negative binomial model) and GLM, respectively, were also used; in this case, tests were performed both taking all digestive tract as a whole and by organs (stomach, pyloric caeca and intestine). To test possible effects of anthropogenic fibres in values of parasite infection (total abundance and for each taxonomic group) non-parametric Spearman's correlation test were performed. Since no relationship between anthropogenic fibres affecting parasite infection were detected (see results below) and as parasite infection can also affect fish health indices, the number of parasites (nPAR) was considered as explanatory variables to analyse the relationships between the anthropogenic fibres and the health biological indices. A multivariate ordination method was used in Xlstat (version 2019.21.3.62256 http://www.xlstat.com). Redundancy analysis (RDA) is a multivariate method appropriated to test or visualize correlations or covariances between the response and explanatory variables, so modelling a cause-effect relationship. RDA was used with 500 Monte Carlo significance permutation tests to identify any possible tendency between nAF, TLAF and number of parasites (nPAR) as explanatory variables and the fish health indices K, HSI, GSI and FULL as response variables. Finally, to assess the possible effect of AFs in the fish health in biological indices as K, HSI, GSI, FULL and SL, Pearson's correlation test and non-parametric Spearman's correlation test (when normality was not satisfied) were performed.

3. Results

310 3.1. Ingestion of Anthropogenic items in *M. barbatus*

311 A total of 167 AFs were found in the digestive tract of *M. barbatus*. In addition, 7 fragments (size
312 range = 0.12 – 0.72 mm, mean length = 0.32mm (SD = 0.20)) were also found in stomach of five
313 distinct fish: five fragments in Barcelona 2018 and one at each Barcelona 2007 and Blanes 2018.
314 Due to their small number they were not considered in subsequent analyzes. Mean AFs length
315 was 2.50 mm (SD = 2.24), ranging 0.37 mm to 14.80 mm. The four clusters based on partitioning
316 around medoids per individual AFs were: Small (S): ≤ 1.9 mm; Medium (M): from 2 to 3.9 mm;
317 Large (L): from 4 to 7.3 mm and Extra Large (XL) ≥ 7.3 mm (Fig. 2). The most abundant size was
318 small (S = 53.45%) (X^2 = 14.129, p = 0.02), followed by medium (M = 34.48%) while less abundant
319 were large and extra-large, (L = 8.62%; XL = 3.45%, respectively). Half of the analyzed red mullets
320 contained AFs in their digestive tract (59 out of 118): 28 and 95 from Barcelona (2007 and 2018
321 respectively), and 51 red mullets from Blanes 2018. The mean number of AFs per individual (nAF)
322 was 1.48 (SD = 1.98), and the mean TLAF was 3.55 mm/individual (SD = 6.17). No significant
323 differences were found (p > 0.05) in the number of AFs at different parts of the digestive tract.
324 The smallest TLAF were found in caeca (K-W = 7.025, p = 0.027), followed by the intestine and
325 stomach (no significant differences). According to fish size and ingestion of AFs, significant
326 positive correlation between nAF and SL were found (X^2 = 8.689, p= 0.003) but not for TLAF.
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329 3.2. AFs characterization
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331 The 167 AFs were classified into five distinct typologies according to GA and MA:
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333 Typology 1 (T1): Prevalence: 10.69 %, size range: 0.90-5 mm. GA: Cellulosic-like fibre easily
334 deformable with circular section shape with maximum thickness in the middle of the fibre that
335 narrows until pointed ends (Fig 3, Typology 1, left) or long and smoothly striated flat fibres with
336 angle-shaped folds (Fig 3, Typology 1, right); transparent fibres, never dyed, slightly birefringent
337 / iridescent. MA: From solid to clearly frayed edges; both crystalline and amorphous regions,
338 without any pattern. A remarkable quick laser burnout was observed during Raman spectroscopy
339 in samples of this typology. All samples shared similar spectra and were identified as cellulose
340 (HQI 75,32%) (Fig. 4C).

Typology 2 (T2): Prevalence: 46.10%, size range: 0.37-9.30 mm, GA: Curly cotton-shaped fibres with flat section, from clearly to slightly twisted; straight, frayed or with breakages ends; sometimes angle-folded; from translucent (Fig. 3, Typology 2, left) to deep blue dyed (Fig. 3, Typology 2, right). MA: non or slightly birefringent flat twisted fibre, solid edges. Spectra obtained in colourless samples of this category showed a similar pattern and 80% were identified as cellulose (HQI > 70 – 80%) (Fig. 4C). When dyed, pigment was identified in 71.43% of samples which corresponded to Indigo dye spectra (HQI > 80%) (Fig. 4C-E).

Typology 3 (T3): Prevalence: 31.14%, size range: 0.50-14.80 mm, GA: Rigid fibre with circular section and solid edges without fraying; if short usually hook shaped; frequently interrupted by molten flat areas of squashed appearance; ends with similar molten flat areas (Fig. 3, Typology 2, left) or club-shaped ends with a splintered breakage appearance (Fig. 3, Typology 3, right); from transparent to yellowish fibres or slightly dyed (red, blue, yellow or green); crystalline without amorphous regions; MA: Discontinuities with grain appearance all along the fibre. Spectra of all samples (100%) analysed with Raman scattering from these typologies were identified as polyethylene terephthalate (PET) (HQI ≥ 90) (Fig. 4B).

Other fibres: Prevalence: 11.38%, size range: 1.10-6.30 mm. Other minority AFs found, that did not obey any of the previous descriptions were grouped into this category. No conclusive coincidences were found those spectra against reference spectra.

Raman spectroscopy corroborated the correct visual classification by fibre morphology, since in all analysed cases fibres from the same category share similar spectra. Raman spectroscopy together with visual classification allowed identifying 56.79% of fibres as cellulose based (T1 and 2), and 31.14% of fibres as PET (T3), which corresponds to a total of 87.93% of AFs characterized and identified.

3.3. Differences in AFs between a decade gap

A significant increase was detected in the number of fish containing AFs between 2007 (29%) and 2018 (74%) in Barcelona ($X^2 = 15.157$, $p < 0.001$) (Fig. 1, Table 2). The increase was also significant regarding the number of the AFs (nAF, $X^2 = 26.286$, $p < 0.001$) and their total length (TLAF, $F_{81,1} = 15.466$, $p < 0.001$). None of these differences were associated to fish size (SL). No significant differences were found in the proportion (%) of AFs by class size between 2007 and

2018. Regarding typologies, T3 (PET) had higher values in 2018 (40%) respect 2007 (20%) but X-squared test (X^2) was not significant due to the low sample size. In contrast, T2 (Cellulose) decreased significantly in 2018 ($X^2 = 6.847$, $p = 0.009$).

3.4. AFs geographical variability

No significant differences in prevalence of fish containing AFs were detected between localities sampled in 2018 (Fig. 1, Table 2). However, AFs in Barcelona were both significantly more abundant (nAF, $X^2 = 4.747$, $p = 0.029$) and larger (TLAF, $F_{68,70} = 9.684$, $p = 0.003$; large-sized AFs in Barcelona, $X^2 = 4.658$, $p = 0.033$; small-sized AFs in Blanes: $X^2 = 9.974$, $p = 0.002$). None of these differences were associated to fish size (SL). No significant differences were found among typologies.

3.5. Effect of AFs in fish health

Indices of fish health status (HSI, GSI, K and FULL) are shown in Table 1. Differences between years were found for K that was significantly lower in 2018 ($F_{82,1} = 9.941$, $p < 0.005$). FULL and GSI showed significant higher values in 2018 but associated to fish size ($X^2 = 4.057$, $p = 0.044$ and $X^2 = 6.385$, $p < 0.05$). Regarding to geographical comparison, only GSI was significantly lower ($X^2 = 8.640$, $p = 0.003$) in Barcelona (no association with fish size was detected). Spearman's test on nAF and TLAF did not show any correlation ($p > 0.05$) between AFs and biological indices (HSI, GSI, K and FULL).

No relevant histopathological alteration was found in any organ of the analysed fish, except for cysts of unknown ethology (CUEs) observed in gills. CUEs consisted on cysts located mainly within gill filament or lamellae, most of the times surrounded by cartilaginous tissue. Significant higher prevalence of CUEs was detected in Barcelona 2007 ($X^2 = 5.369$, $p < 0.05$) and in Blanes ($X^2 = 8.929$, $p < 0.05$) respect to Barcelona 2018. No correlations between CUEs with fish size (SL) and anthropogenic fibres (nAF and TLAF) were found (GZM, $p > 0.05$). Significant higher number of MMC in spleen were detected in fish from Barcelona in 2018 ($X^2 = 16.543$, $p < 0.001$) than in 2007, but no in their size (MA.MMC: $p > 0.05$). No significant differences were found for size or number of MMC between Barcelona and Blanes. No relation between MMC and SL was

found (GZM; $p > 0.05$). Spearman's correlation test did not give any correlation of nMMC nor MA.MMC in relation to anthropogenic fibres (nAF and TLAF).

A total of 2464 parasites were found in 118 *M. barbatus*. The most abundant group were nematodes, followed by digeneans, crustaceans and cestodes (Table 2). Significant higher values of parasite diversity and richness were found in Barcelona 2018 (K-W = 5.293, $p < 0.005$ and K-W = 8.701, $p < 0.005$, respectively) compared to 2007. Significant higher total abundance of parasites ($X^2 = 6.6768$, $p = 0.009$) was also found in 2018, especially due to the higher abundance of nematodes ($X^2 = 14.551$, $p < 0.001$). Regarding geographical comparison, higher values of parasite diversity and richness (K-W = 13.909, $p < 0.005$ and K-W = 11.130, $p < 0.005$, respectively) were found in Barcelona compared to Blanes. However, significant higher abundance of parasites was found in Blanes ($X^2 = 4.200$, $p = 0.040$), again due to the higher abundance of nematodes ($X^2 = 4.478$, $p = 0.034$). No significant differences by year or locality were found for the rest of the taxonomic groups of parasites (Table 2). A positive correlation was found between the number of parasites and the SL of fish ($X^2 = 4.747$, $p = 0.029$). No correlation (Spearman's correlation test, $p > 0.05$) was found between the total number of metazoan parasites (or each taxonomic group) and AFs (nAFs and TLAF), nor considering only parasites within digestive tract (GZM, $p > 0.05$).

RDA explained 99.71% of variability by the first two axis. A positive relation was observed between the number of parasites and fullness, as well as a weak negative relation between the number of parasites, nAF and TLAF with the condition index K and HSI (Fig. 5), although none of them were significant.

4.Discussion

This study provides new information on the prevalence and abundance of AFs in wild organisms and assesses the potential impact of this pollutant on the health status of fish. Half of the red mullet analysed in the present study presented AFs (plastic and non-plastic fibres) in their digestive tract, slightly higher than the prevalence reported by Bellas et al., (2016) in the same area (prevalence of 33% in Barcelona coast). However, similar values are found when comparing the average number of AFs/ind (1.48 AFs/ ind (SD = 1.98) in our study versus 1.75 (SD = 1.14) MP / ind) found by Bellas et al., (2016). This comparison has to be considered in caution as Bellas

et al., (2016) does not report non-synthetic fibres. Differences in MPs among other areas in both basins of the Mediterranean Sea are usual: Spanish Mediterranean Coast (prevalence 18.8%, mean = 1.9 (SD = 1.29) MPs/ind., Bellas et al., 2016),Turkish shores (66% of fish ingested a mean of 2.12 (SD = 1.39) MPs/ind., Güven et al., 2017), Adriatic Sea (64%, mean = 1.57 (SD = 0.78) MPs/ind., (Avio et al., 2015), Ionian Sea (32%, mean = 0.5 (SD = 0.20) MPs/ind., (Digka et al., 2018). Alomar et al. (2017) found slightly lower values in the sister species *M. surmuletus* in the nearby geographical area (Balearic Sea, 27.3% and mean number of 0.42 MP/ind). Differences among these studies may greatly depend in the methodology used in each case. Most of these studies include only plastic items, and some of them also included other items different than fibres (e.g. microbeads or fragments), which increase their number of MP by individual. Our results include mainly AFs, their detection is based on visual screening, and include plastic and non-plastic fibres, resulting in more than half of AFs being non-plastic fibres. Instead, most of the previous studies have focused on exclusively plastic fibres, underestimating the number of AFs and making difficult to compare these values among studies. Most of the studies based on MPs, either in purpose discard polymers different than plastics, or generally use digestion and density separation methods to separate plastic material from organic matter by (Hidalgo-Ruz et al., 2012). This digestion of organic matter with KOH, NaOH or H_2O_2, or incubation at high temperatures in most cases disintegrate cellulosic fibres (Dehaut et al., 2016) resulting in a neglection of these. Also, digestions may affect to the artificial polymer integrity .

4.1. AFs characterization

Fibres are the major abundant shape found in *M. barbatus* in the present study (97% of debris were fibres) which agrees with other studies in the Mediterranean areas (Avio et al., 2015; Giani et al., 2019; Güven et al., 2017). There is only a single study in *M. barbatus* that reported an opposite trend in Northern Ionian Sea (Digka et al., 2018), were fragments seemed to be the most important shape. Regarding size, the largest fibre found in our study is 14.8 mm, much larger than those reported in previous studies (sizes up to 3 mm or 5 mm) (Bellas et al., 2016; Digka et al., 2018). However, most of the fibres found in our fish were shorter than 4 mm (88%), which fit with microplastic / microfibre size definition (MPs < 5 mm, Hartmann et al., 2019). The size of the fibres in this definition is based on their length, so fibres longer than 5 mm are not usually considered. Nonetheless, longer fibres (> 5mm) found in guts usually appear entangled or folded

462 on themselves or with other fibres (Carreras-Colom et al., 2018; Lusher et al., 2013), and may
463 occupy similar volume in the stomach to shorter ones or other microplastic. Thus, this definition
464 by size should be regarded with caution in ecological and health assessment studies of
465 organisms. For that reason, we also used the measure of the addition of the fibre length of AFs
466 observed in the digestive tract of each individual, TLAF (3.51 mm / ind), which give a more realistic
467 value of the volume occupied by these fibres in the digestive.
468
469 The results presented in this study demonstrate the importance of an accurate visual approach,
470 that enables to discriminate between distinct fibre types (88% of AFs can be classified) and to
471 infer their possible composition and origin, prior to analyses based on spectroscopic techniques.
472 As Robertson et al (2017) point out, AFs can be characterized visually by many morphological
473 parameters which give them a high degree of variability, such as the colour, diameter, shape,
474 particles included in the fibre, ends-shape, cross-sectional shape, and size. This enables the
475 identification of natural fibres (animal, vegetal or mineral), man-made fibres derived from natural
476 polymers, such as cellulose (the regenerated fibres), and true synthetic fibres synthesized from
477 simple organic chemicals found in coal and oil (Robertson et al., 2017; Stanton et al., 2019). For
478 example, colour is not useful to distinguish between natural (e.g., cotton) and artificial fibres (e.g.,
479 viscose) (Remy et al., 2015), but delustrants (eg. paraffin wax or titanium dioxide) enable to
480 identify only the non-natural ones (Robertson et al., 2017). The latter are additives used during
481 the manufacturing process to reduce the brightness of the resulting polymer and they are
482 perceived under the microscope as inclusions of grain appearance (Robertson et al., 2017) such
483 those observed in T3 fibres. Raman spectroscopy later confirm the non-natural composition (PET)
484 in this type of fibres. The molten areas found in T3 fibres have been well described for synthetic
485 textile fibres as polyesters (De Wael et al., 2011; Lepot et al., 2008). These parts are formed by
486 ironing or during industrial fabrication process (De Wael et al., 2011) and is another characteristic
487 feature that enable their classification as a thermoplastic, in agreement with its PET composition.
488
489 Our results found 57% cellulosic fibres versus 31% PET in the gastrointestinal tract of *M.*
490 *barbatus*, in agreement with the fact that cellulosic fibres are just dominant over synthetic
491 polymers in some Mediterranean marine environments (Sanchez-Vidal et al., 2018). Cellulose
492 based fibres are one of the most used fibres in textile industry (Textile World, 2015), which include
493 natural fibres such as cotton and man–made regenerated fibres produced from dissolving

494 cellulose-based raw material, such as viscose or rayon. Cotton can be recognized by its flattened
495 and twisted appearance (Robertson et al., 2017), as are the fibres grouped in T2 in the present
496 study. This characteristic curly shape comes from the natural twist or convolution formed during
497 natural fibre growth. Moreover, high percentage of indigo blue dye is further detected by Raman
498 spectroscopy analysis in T2 fibres, which is also the most used dye in blue-dyed cotton fibres,
499 called denim fibres, characteristic of jeanswear (De Wael et al., 2011; Grieve et al., 2006; Grieve
500 and Biermann, 1997; Robertson et al., 2017). Due to their morphology and colour, cellulosic fibres
501 classified as T1 do not seem to have a textile origin.
502 The morphology of synthetic fibres and the use of certain dyes in these fibres suggests that most
503 anthropogenic fibres found in our study may have a textile origin (46% of T2, cotton-shaped fibres
504 plus a 31% of T3, PET). It has been well described how textile fibres from washing machines
505 waste effluent travel via wastewater to sewage treatment plants (Dris et al., 2015; Leslie et al.,
506 2017) and end up in the ocean (Browne et al., 2011; Napper and Thompson, 2016). Different
507 habitats accumulate different types of marine debris (Anastasopoulou et al., 2018) due to their
508 density. While denser polymers (e.g. PET and cellulose) tend to sink to the seabed, lighter
509 particles are more commonly found floating in pelagic waters (e.g. low-density polyethylene
510 LDPE) (Andrady, 2011). Although some exceptions have been described (Bottari et al., 2019)
511 demersal fish species, such as *M. barbatus,* ingest more denser polymers and lower percentages
512 of lower dense polymers compared to shallower ones (Alomar et al., 2017; Avio et al., 2015).
513 In the Mediterranean continental shelf and deep seafloors, specifically at the same geographical
514 region of the present study, cellulosic fibres are the most abundant (80%), followed by PET
515 (12.9%), acrylic (polymethyl methacrylate), polyamide, polyethylene, and polypropylene
516 (Sanchez-Vidal et al., 2018), which also agree with fibre importance found in the digestive tract
517 of *M. barbatus* in the present study. PET is the most abundant synthetic polymer fibre found in
518 *Mullus surmuletus* in Mediterranean Sea (Alomar et al., 2017). However, several studies in the
519 Mediterranean Sea have also found non-plastic fibres mainly based on cellulose in different fish
520 species (Compa et al., 2018; Savoca et al., 2019), as in the present study. As explained before,
521 non-plastic fibres could be easily mistaken with plastic (Remy et al., 2015), so its importance in
522 marine environments could be greater that what is known so far.
523
524 4.2. Differences between a decade gap

525 The number of fishes containing AFs in Barcelona is higher in 2018 respect the same area in
526 2007. Not only fibre prevalence, but also nAF and TLAF values were 5 and 4 times higher in 2018
527 than 2007, respectively.
528 The presence of AFs in the gastrointestinal tracts of the studied fish from 2007 demonstrates how
529 the ingestion of this type of debris is not a new phenomenon in the NW Mediterranean Sea. The
530 high increase not only in its prevalence but also in the number of the AFs in 2018, could be the
531 reflection of the accumulation of this debris in marine environment (Avio et al., 2015) along the
532 last decade. However, this interpretation should be taken with caution because differences
533 between years could be also attributed to sporadic oscillations of different events in the same
534 area at different times, resulting in a punctual increase in AFs. For example, rain regime in
535 Barcelona area was especially abundant during 2018 (984.2 mm) compared to mean value (580.6
536 mm) over the past 10 years (Meteocat 2019), which may result in an exceptional increase of river
537 discharges in that area. So, it is not possible to clearly establish an upward trend over the years.
538 The results seem to indicate a change between the years of the study in the levels of
539 contamination of cellulosic fibres towards PET fibres, since lower significant values are detected
540 in T2 (characterised as cellulose with a possible textile origin) in 2018. Changes in textile
541 demands of recent decades may support this tendency because, currently, synthetic fibres ahead
542 by polyesters like PET dominate the global fibre market and have overtaken the natural cotton,
543 declining its production year-after-year (Carr, 2017).
544
545 4.3. Geographical variability
546 AF abundance are higher in Barcelona than in Blanes, and despite not being significant, the
547 number of fish containing AFs are also higher in Barcelona. In addition, higher values of TLAF
548 (three times higher in Barcelona respect to Blanes) may be due to both, an increase in the number
549 of ingested fibres, and the larger size of these fibres. The high levels of AFs found in Barcelona
550 compared to Blanes may result from the specific level of anthropization of the surrounding land
551 areas, the level of water discharge by rivers and the specific features of the continental shelf. The
552 main inputs of plastic to the marine environment come from industrialized and densely populated
553 coastal areas (Andrady, 2011; Derraik, 2002; Jambeck et al., 2015) such the area surrounding
554 Barcelona. Several pathways of dispersion of microfibres into the marine environment have been
555 described, such as the atmospheric fallout (Dris et al., 2017), the wastewater treatment plants
556 and the storm-water runoff (Wagner and Lambert, 2018). The latter are considered as a critical

input of synthetic fibres to the aquatic environment via river basins and finally discharging in the ocean (Horton et al., 2017b; Murphy et al., 2016). Both presently studied areas are under the influence of several rivers which drain water from urbanized areas, so the presence of AFs could be expected (de Haan et al., 2019; Sanchez-Vidal et al., 2013). Even though, the continuous river regime and the higher level of flow in Barcelona compared to Blanes may explain the higher levels of AFs found in this area. Moreover, the submarine canyon of Blanes, a great canyon that drives sediment transport into the deep-sea and minimizes sediment deposition in southern continental shelf, loses part of sediment discharges and pollutants from this river. Northern shelf of Blanes is already a starved-sediment natural area due the same effect of northern submarine canyons (Durán et al., 2014). In addition to river discharge, estuarine and river benthic sediments influence the microfibre accumulation (Horton et al., 2017a; Simon-Sánchez et al., 2019), that finally are retained in the continental shelf (Sanchez-Vidal et al., 2018). Muddy and fine grained sediments, like those of Llobregat and Besòs rivers, may have a greater retention of pollutants (Van Cauwenberghe et al., 2013) in contrast to sandy sediments, like the sandy coarse immature sediments poured by Tordera river (Durán et al., 2014). Hence, the continental shelf, where red mullet inhabits, off Barcelona may accumulate higher amounts of AFs compared to Blanes shelf. The absence of significant differences in fibre composition between Barcelona and Blanes suggests that the two sites are reflecting a similar source or similar contribution in both areas driven by population density. However, higher proportion of T2 were found in Blanes, which may suggest a source of this specific cotton typology in this area.

4.4. Effect of AFs in fish health

In the present study, the levels of ingested AFs in *M. barbatus* from this study do not seem to interfere with feeding activity, nutritional state or reproductive capacity, since no relation is found between the number or size of AFs with fish health condition indicators (HIS, GSI, K, FULL). Differences by year and locality observed in GSI and FULL are most probably related to fish size. The differences of K between years in *M barbatus* from present study may related to fish biology (e.g. reproduction events) which can show changes between years due the fact that they are strongly dependant to environmental variables. Higher values of GSI as a consequence of gonad development are carried out from fish lipid reserves, which results in a reduction in K. To restore this loss off energy feeding intensity increases which is reflected in higher values of fullness. Although in wild pelagic species, such as *Sardina pilchardus,* lower values of K has been

attributed to higher values of AF ingestion, although this is not was clear since other variables such as latitude seems to be influencing (Compa et al., 2018). In fact, effects of MP on body length or condition factors are ambiguous, as both reduced and increased levels are reported (Kögel et al., 2020).

It is known that the ingestion of fibres, both plastic and non-plastic ones, due to their shape and its physical performance, can result on agglomerations of fibres blocking the digestive tract (Lusher et al., 2013). This is an effect demonstrated in some wild crustaceans, probably enhanced by the characteristic anatomy of their digestive (Carreras-Colom et al., 2018; Welden and Cowie, 2016). However, in wild fish these effects are not usual. Entangled and folded fibres are observed in *M. barbatus* in the present study but without producing balls, probably due to the small number of AFs ingested and the shape of the digestive tract in this species. The digestive tract of red mullet does not present too many modifications, apart from the pyloric caeca which present several digitations (Le Pommelet and Silan, 1998). Smaller fibres were found in this part of the intestine but, as other authors suggest (Grigorakis et al., 2017), they seem to be normally egested (without accumulation) since no differences in number of fibres are observed along the entire digestive tract.

Histopathological alterations attributable to AFs ingestion are not found in present study. CUEs is the only histological alteration found in *M. barbatus,* with similar prevalence described in previous studies in NW Mediterraean Sea (Carreras-Aubets et al., 2011). Although the presence of this alteration has been suggested to be linked to environmental pollution (Carreras-Aubets et al., 2011; Munday and Brand, 1992), no relation with AFs are found in our study. Splenic MMC are known to increase in size and frequency due to multiple factors such as fish age, infectious processes and cell destruction, recycling or storage of endogenous and exogenous materials and also under environmental chronic stress (Agius and Roberts, 2003). In this study, the change in the number of splenic MMC between years is not related to AFs.

However, histopathological alteration due to the ingestion of anthropogenic particles are commonly reported after artificially exposures, under laboratory conditions (Kögel et al., 2020; Limonta et al., 2019). It is important to notice that the concentration of MP used in laboratory analysis is usually extremely higher than natural condition, and the concentration seems to be one of the most important factors when causing harm to fish (Kögel et al., 2020). In these studies, the most frequent alterations reported in fish associated to MP (>10µm) are reduction in the activity, physiological stress and hormonal dysregulation together with intestinal damage

(Kögel et al., 2020). Alterations in digestive tract epithelium in direct contact with ingested AFs may be expected, but it is not the case in *M. barbatus*. This result agrees with other authors (Batel et al., 2020; Jovanović, 2017), which point to ingested MPs pass along the intestinal lumen without causing harm. Although some authors detect plastics of up to 0.6 mm in liver (Avio et al. 2015), it is highly unlikely that MPs or AFs as large as those in this study are able to pass through the intestinal barrier, and reach other organs, but smaller AFs (usually less than 100-150µm) or even their additives (often toxics) may do. Some of the alterations described in liver due to exposure to MP (and associated toxics or pollutants) include glycogen depletion, fatty vacuolation, inflammatory infiltration or necrosis (Lu et al., 2016; Rochman et al., 2013)(Sures, 2001). However, none of these signs is observed in fish from present study, which again indicate that AFs ingested by *M. barbatus* are excreted without any adverse effects.

Parasites of red mullet have been well described in the Mediterranean Sea, as well as its variations in relation to environmental pollutants (Carreras-Aubets et al., 2012, 2011). In the present study, higher parasite abundance, richness and diversity are observed in 2018 and in Blanes (if compering locations), but in relation to fish size, especially due to high numbers of nematodes. This is a common phenomenon associated to host longevity and space available especially for long-living larval nematodes (González and Acuña, 2000). Parasites are commonly used as early warning bioindicators for environmental and fish health assessment (eg. Lafferty, 1997; Marcogliese, 2005). However, there are no studies relating variations in parasites communities with plastics. In the present study the ingestion of AFs in *M. barbatus* do no seems to have effects in parasite descriptors, nor vice versa. Thus, the parasites within the digestive tract of *M. barbatus* do not seem to interfere in the retention and accumulation of MP, which disagree with the hypothesis of Hernandez-Milian et al. (2019).

5.Conclusions

The ingestion of AFs in *M. barbatus* is not a new phenomenon in the Mediterranean Sea since it is already detected 10 years ago. Visual fibre characterization, corroborated by spectroscopic techniques, allow identifying distinct fibres typologies of both synthetic polymers and cellulose. The most abundant fibres were cellulosic-based fibres followed by PET fibres, with a possible textile origin, while other typologies were minority. *Mullus barbatus* can show spatial changes in

the number of ingested AFs and also between years, but in no cases health status indicators (condition indices, histopatological and parasitological analyses) reflect an effect of AFs ingestion. Although several studies point out the potential negative effect of this kind of pollutants, this study demonstrates that, no damage in health condition can be detected at levels of AFs ingestion in wild fish. Our results reinforce using red mullet as a suitable benthic species for monitoring of this type of marine debris, spatially and among years. Fish with distinct feeding behaviours and living habitats should be also monitored, as AFs might affect them at different ways.

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Figure and table captions:

Fig1. Map of sampling area. Circles (○) indicate sampling stations near Barcelona and triangles (Δ) near Blanes. Black and grey fill colour for summer and spring respectively, (+) indicates 2007 sample stations. Ring graphs indicate prevalence of fish containing anthropogenic fibres in Barcelona 2007 (A), Barcelona 2018 (B) and Blanes 2018 (C). Different numbers and letters show significant differences between years (Barcelona 2007-2018) and localities (Barcelona-Blanes in 2018), respectively ($p<0.05$).

Fig. 2. Levels and typologies of anthropogenic fibres (AFs). Percentage (ring graph) and number (bar graph) of AFs by size at between years A) and spatial scale B). Percentage of the different anthropogenic fibres by

typologies between years C) and spatial scale D). Different letters (a, b) show significant differences in AFs size between localities (Barcelona-Blanes in 2018) ($p<0.05$). Different numbers (1,2) in typologies are expressed significant differences between years (Barcelona 2007-2018) ($p<0.05$). Labels expressed in X axis of figures C and D corresponds to different fibre typologies described in the text: T1 (cellulose), T2 (cotton-shaped cellulose) T3 (PET), and others (unidentified).

Fig. 3. Optical microscope images of anthropogenic fibres (AFs) found in the guts of *Mullus barbatus.* Distinctive features of each typology are shown with detail of the general appearance of AFs in the small box. Typology 1 identified as cellulose, typology 2 as cotton-shaped cellulose, and typology 3 as PET.

Fig. 4. Raman analyses. A) Example of fibre area analysed during Raman spectroscopy in which optical image (scale bar = 100 µm) of fibre selection area (upper image) and Raman image (scale bar = 50 µm) of the selected sampled area (inferior) are shown. The colour scale bar below represents intensity of the integrated spectral band. B-E) Raman scattering spectra of: Cellulose reference spectra compared to Typology 1 (HQI 75,32%) and 2 (HQI > 70 – 80%) compared with cellulose spectra (B), Typology 2 blue fibre spectra (C) , section of maximum intensity of typology 2 blue fibre spectra compared to Indigo dye spectra (HQI > 80%) (D) and typology 3 (HQI ≥ 90) compared with PET reference spectra (E).

Fig. 5. Redundancy analysis (RDA) between anthropogenic fibres (AFs) and parasites with condition indices. Number (nAF) and total length (TLAF) of AFs and the number of parasites (nPARASITES) as explanatory variables respect to the response variables of the fish health indices condition factor (K), hepatosomatic index (HSI), gonadosomatic index (GSI) and fullness (FULL).

Supplementary figure. Custom library spectra obtained by Raman spectroscopy of target materials. High density polyethylene (HDPE), nylon (PA6), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), textile cellulose (rayon), textile cotton, graphite and silicon.

Table 1. Cruise data (station, location, year, season, depth, latitude and longitude) for each sampling site, including the number of fish analysed (n). Mean and standard deviation (SD) of standard length (SL, cm), total weight (TW, g), gonadosomatic index (GSI), hepatosomatic index (HSI), condition factor (K) and fullness (FULL).

Table 2. Anthropogenic fibres, parasite descriptors and histopathological alterations found in *Mullus barbatus*. Mean and standard deviation (SD) of the number (nAFs) and total length (TLAFs) of the anthropogenic fibres found in the digestive system of *Mullus barbatus*. Prevalence (P%) of parasites and Cyst of Unknown Etiology (CUEs), mean abundance (MA) and standard deviation (SD) of parasites and Melanomacrophagic centres (MMC), and mean tissue area (A. Me., μm^2)) and standard deviation (SD) of

MMC. Different numbers and letters show significant differences between years (Barcelona 2007-2018) and localities (Barcelona-Blanes in 2018), respectively ($p<0.05$).

Supplementary table. Prevalence (P%), mean abundance (MA) and standard deviation (SD) of parasite species of each taxonomic group found in *Mullus barbatus*.

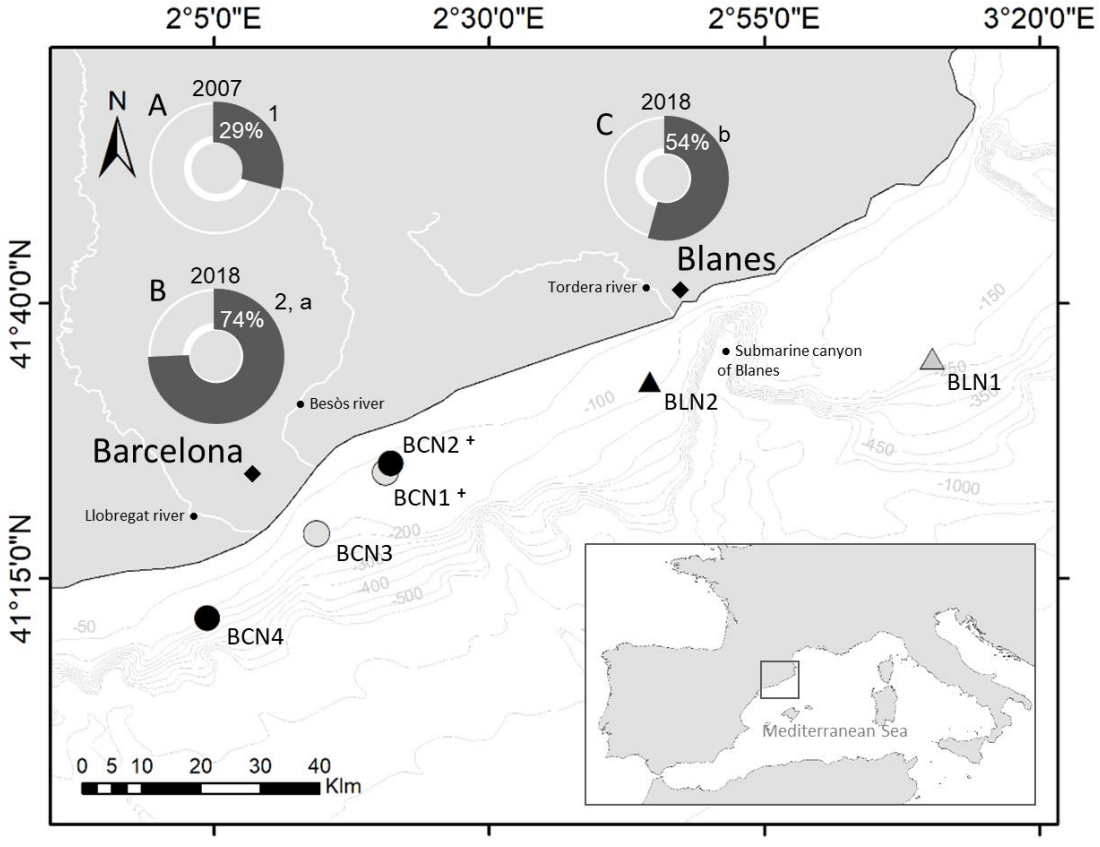


Fig1. Map of sampling area. Circles (○) indicate sampling stations near Barcelona and triangles (Δ) near Blanes. Black and grey fill colour for summer and spring respectively, (+) indicates 2007 sample stations. Ring graphs indicate prevalence of fish containing anthropogenic fibres in Barcelona 2007 (A), Barcelona 2018 (B) and Blanes 2018 (C). Different numbers and letters show significant differences between years (Barcelona 2007-2018) and localities (Barcelona-Blanes in 2018), respectively ($p<0.05$).

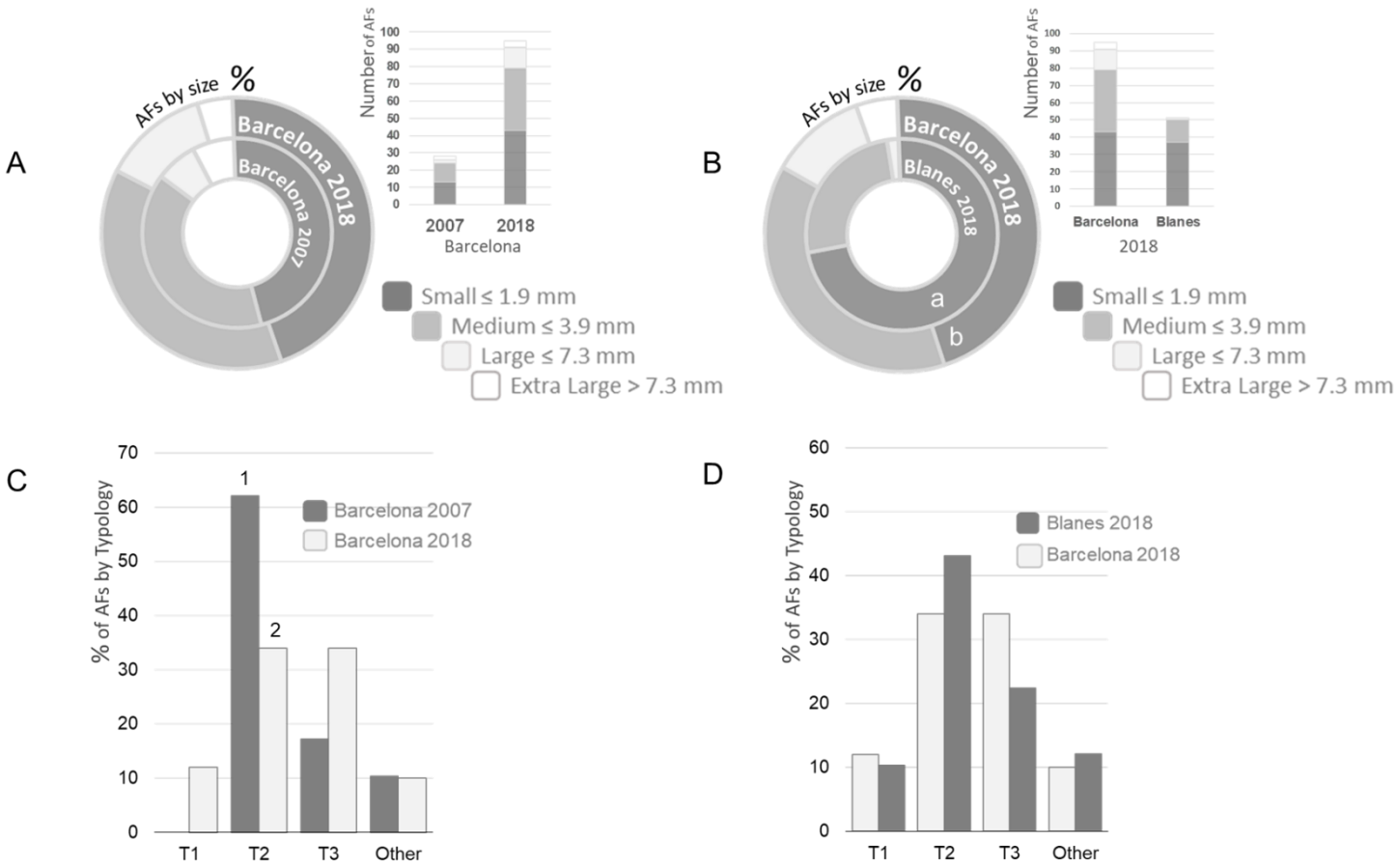


Fig. 2. Levels and typologies of anthropogenic fibres (AFs). Percentage (ring graph) and number (bar graph) of AFs by size at between years A) and spatial scale B). Percentage of the different anthropogenic fibres by typologies between years C) and spatial scale D). Different letters (a, b) show significant differences in AFs size between localities (Barcelona-Blanes in 2018) ($p<0.05$). Different numbers (1,2) in typologies are expressed significan differences between years (Barcelona 2007-2018) ($p<0.05$). Labels expressed in X axis of figures C and D corresponds to different fibre typologies described in the text: T1 (cellulose), T2 (cotton-shaped cellulose) T3 (PET), and others (unidentified).

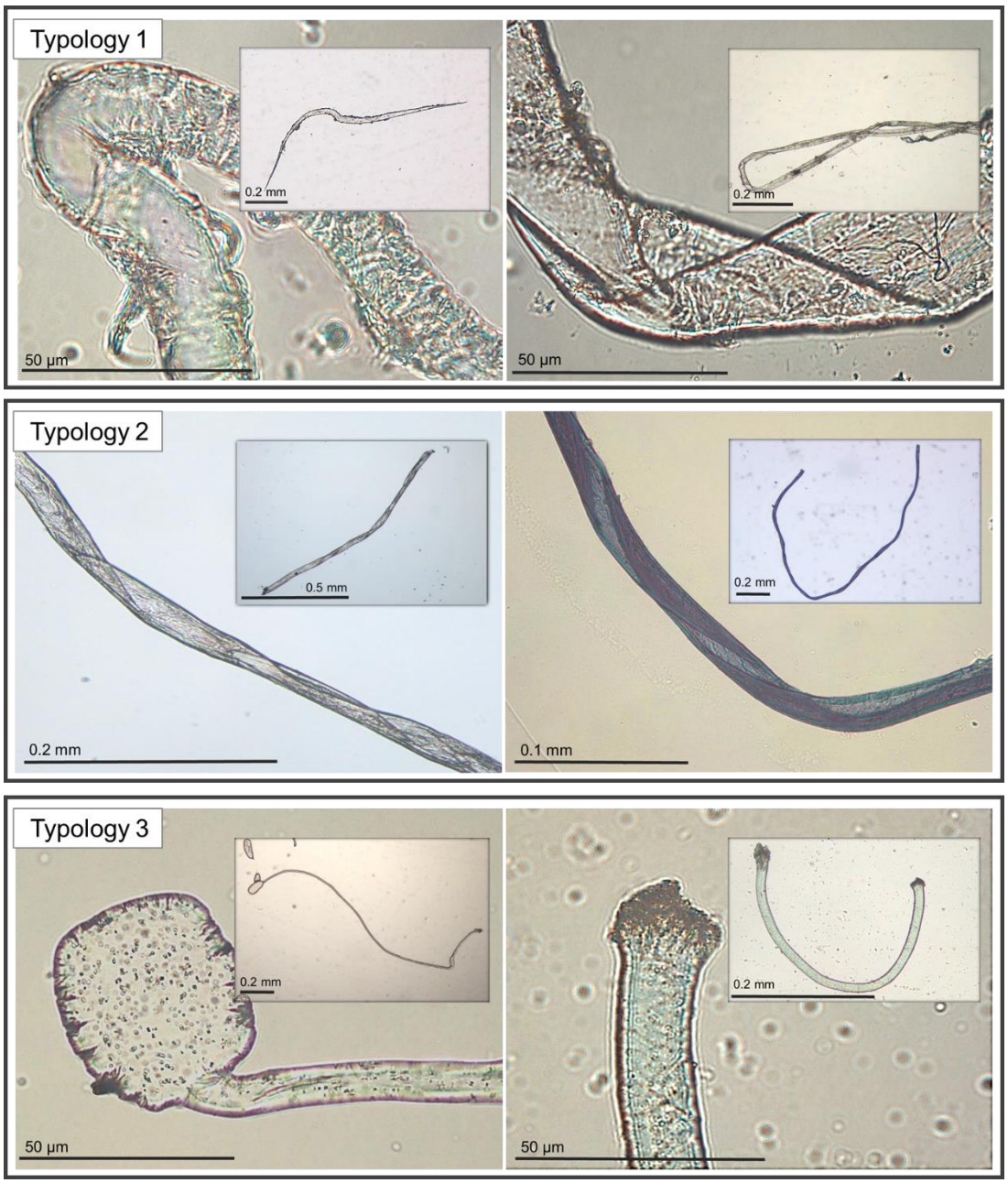
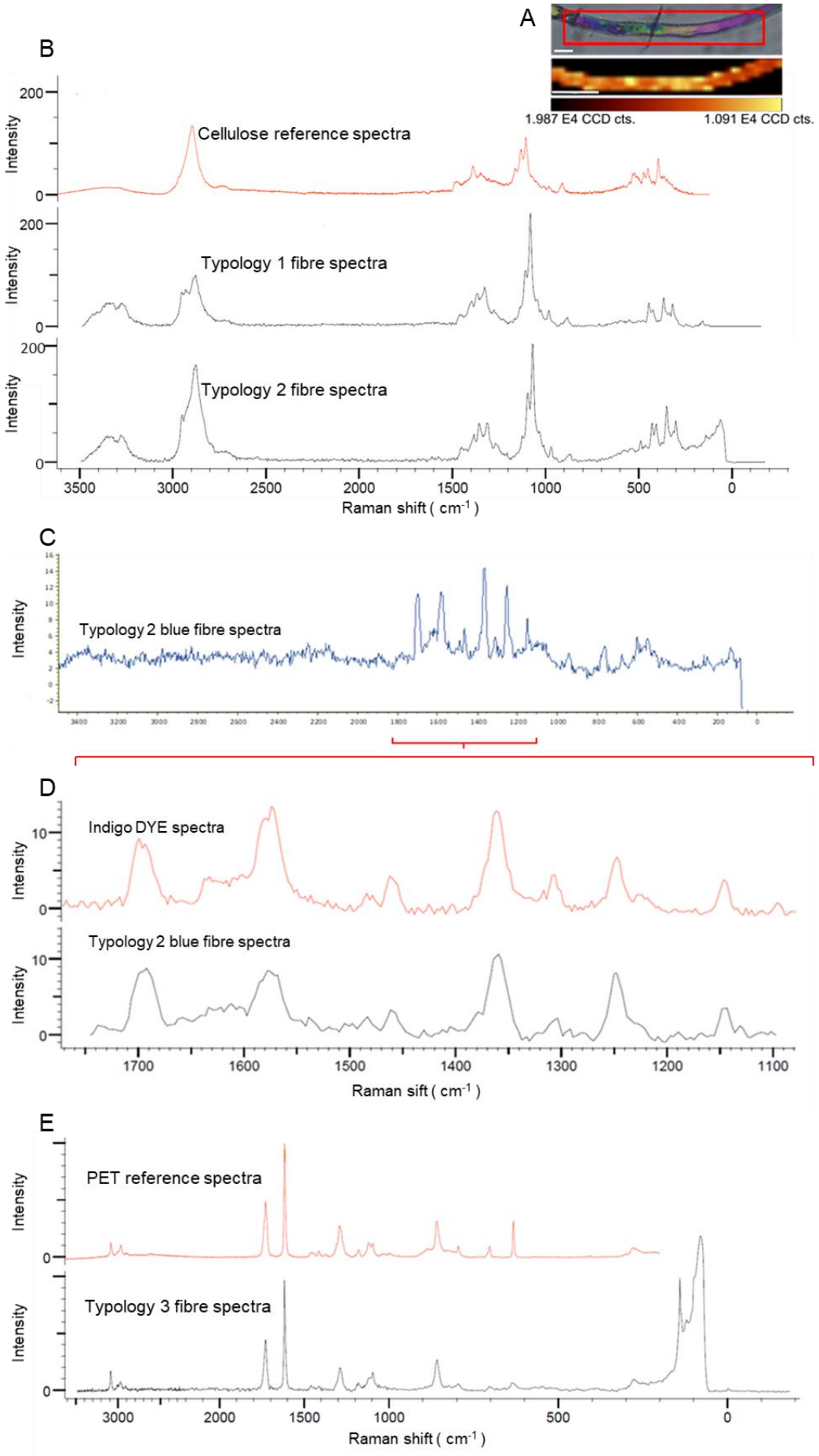


Fig. 3. Optical microscope images of anthropogenic fibres (AFs) found in the guts of *Mullus barbatus.* Distinctive features of each typology are shown with detail of the general appearance of AFs in the small box. Typology 1 identified as cellulose, typology 2 as cotton-shaped cellulose, and typology 3 as PET.


A
1.987 E4 CCD cts.
1.091 E4 CCD cts.
B
Cellulose reference spectra
Typology 1 fibre spectra
Typology 2 fibre spectra
Intensity
Raman shift (cm-1)
C
Typology 2 blue fibre spectra
D
Indigo DYE spectra
Typology 2 blue fibre spectra
Raman sift (cm-1)
E
PET reference spectra
Typology 3 fibre spectra
Raman shift (cm-1)

1112

Fig. 4. Raman analyses. A) Example of fibre area analysed during Raman spectroscopy in which optical image (scale bar = 100 µm) of fibre selection area (upper image) and Raman image (scale bar = 50 µm) of the selected sampled area (inferior) are shown. The colour scale bar below represents intensity of the integrated spectral band. B-E) Raman scattering spectra of: Cellulose reference spectra compared to Typology 1 (HQI 75,32%) and 2 (HQI > 70 – 80%) compared with cellulose spectra (B), Typology 2 blue fibre spectra (C) , section of maximum intensity of typology 2 blue fibre spectra compared to Indigo dye spectra (HQI > 80%) (D) and typology 3 (HQI ≥ 90) compared with PET reference spectra (E).

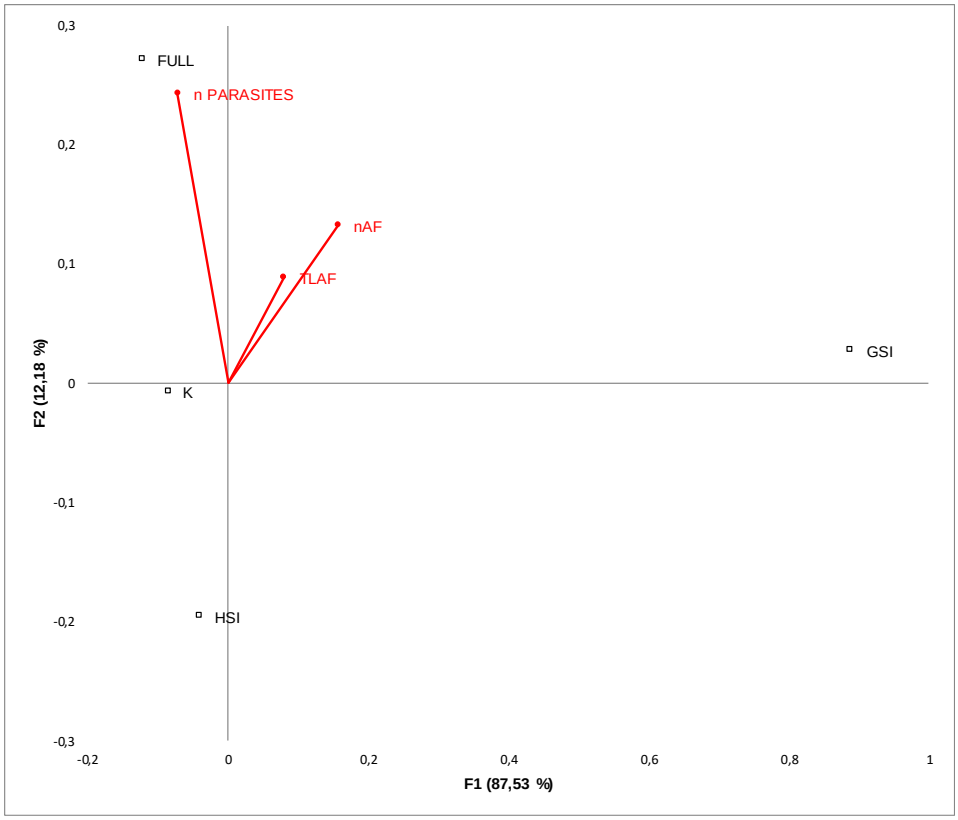


Fig. 5. Redundancy analysis (RDA) between anthropogenic fibres (AFs) and parasites with condition indices. Number (nAF) and total length (TLAF) of AFs and the number of parasites (nPARASITES) as explanatory variables respect to the response variables of the fish health indices condition factor (K), hepatosomatic index (HSI), gonadosomatic index (GSI) and fullness (FULL).

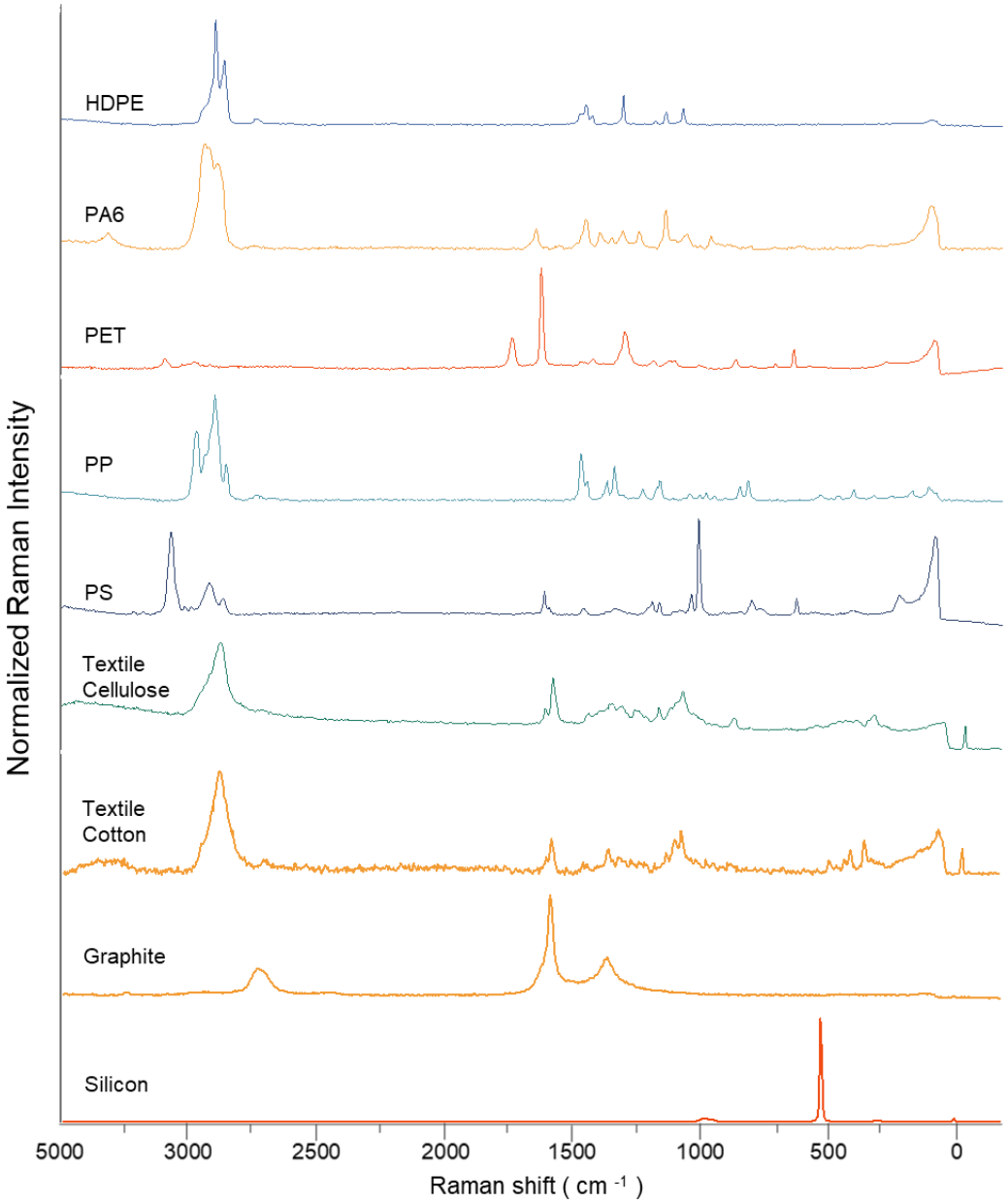
Table 1. Cruise data (station, location, year, season, depth, latitude and longitude) for each sampling site, including the number of fish analysed (n). Mean and standard deviation (SD) of standard length (SL, cm), total weight (TW, g), gonadosomatic index (GSI), hepatosomatic index (HSI), condition factor (K) and fullness (FULL).

Station	Location	Year	Season	Depth	Lat.	Long.	n	SL (SD)	TW (SD)	GSI (SD)	HSI (SD)	K (SD)	Full (SD)
BCN1	Barcelona	2007	Spring	62 m	41°24'35.10"N	2°20'31.80"E	19	9.46 (0.79)	16.20 (3.91)	1.48 (0.51)	1.71 (0.46)	1.68 (0.12)	0.27 (0.22)
BCN2	Barcelona	2007	Summer	62 m	41°25'25.92"N	2°21'1.68"E	29	12.60 (1.90)	41.67 (22.55)	2.21 (1.60)	1.74 (1.48)	1.75 (0.20)	0.43 (0.37)
BCN3	Barcelona	2018	Spring	93 m	41°19'1.56"N	2°14'16.44"E	15	12.08 (1.31)	30.27 (10.15)	2.19 (1.44)	1.39 (0.47)	1.49 (0.13)	0.24 (0.25)
BCN4	Barcelona	2018	Summer	106 m	41°11'21.54"N	2° 4'23.16"E	20	13.99 (0.86)	51.17 (9.25)	1.72 (1.82)	1.78 (0.47)	1.67 (0.13)	0.69 (0.51)
BLN1	Blanes	2018	Spring	130 m	41°34'59.52"N	3°10'13.62"E	15	14.93 (1.23)	62.70 (17.14)	4.84 (2.76)	1.85 (0.77)	1.57 (0.12)	0.42 (0.24)
BLN2	Blanes	2018	Summer	110 m	41°32'51.36"N	2°44'34.80"E	20	17.26 (1.72)	49.82 (14.94)	3.41 (3.59)	1.90 (0.78)	1.65 (0.13)	0.70 (0.60)

Table 2. Anthropogenic fibres, parasite descriptors and histopathological alterations found in *Mullus barbatus*. Mean and standard deviation (SD) of the number (nAFs) and total length (TLAFs) of the anthropogenic fibres found in the digestive system. Prevalence (P%) of parasites and Cyst of Unknown Etiology (CUEs), mean abundance (MA) and standard deviation (SD) of parasites and Melanomacrophagic centres (MMC) and mean tissue area (A. Me., μm^2) and standard deviation (SD) of MMC. Different numbers and letters show significant differences between years (Barcelona 2007-2018) and localities (Barcelona-Blanes in 2018), respectively ($p<0.05$).

Year	2007			2018					
Locality	Barcelona			Barcelona			Blanes		
ANTHROPOGENIC FIBRES		**Mean**	**(SD)**		**Mean**	**(SD)**		**Mean**	**(SD)**
nAF		0.56 [1]	(1,03)		2.77 [2 a]	(2,43)		1.46 [b]	(1,79)
TLAF		1.65 [1]	(3,75)		7.64 [2 a]	(9,18)		2.44 [b]	(3,04)
PARASITES	**P%**	**MA**	**(SD)**	**P%**	**MA**	**(SD)**	**P%**	**MA**	**(SD)**
METAZOA									
CNIDARIA									
Myxozoa	16.67	-	-	8.57	-	-	17.14	-	-
NEMATODA	87.50	7,13 [1]	(6.97)	100	17,29 [2 a]	(11.35)	100	29 [b]	(32.81)
PLATYHELMINTHES									
Trematoda									
Digenea	83.33	3.06	(2.92)	77.14	3.54	(4.07)	82.86	5.51	(6.34)
Monogenea	2.08	0.02	(0.14)	5.71	0.06	(0.24)	-	-	-
Cestoda	2.08	0.02	(0.14)	5.71	0.11	(0.32)	5.71	0.17	(0.86)
ARTHROPODA									
Copepoda	-	-	-	8.57	0.17	(0.62)	2.86	0.03	(0.17)
Isopoda	22.92	0.33	(0.72)	2.86	0.02	(0.17)	-	-	-
PROTISTA									
AMOEBOZOA	8.33	-	-	-	-	-	-	-	-
APICOMPLEXA	-	-	-	-	-	-	5.71	-	-
CILIOPHORA	-	-	-	-	-	-	5.71	-	-
Shannon Diversity Index (H')	1.56			1.76			1.27		
Parasites Species Richness	4.10			5.26			3.86		
HISTOPATHOLOGY	**P%**			**P%**			**P%**		
Epitheliocystis	4.17			2.86			-		
Cysts of Unknown Etiology (CUEs)	25 [1]			5,71 [2 a]			34,29 [b]		
	MA	**(SD)**	**A. Me.** **(SD)**	**MA**	**(SD)**	**A. Me.** **(SD)**	**MA**	**(SD)**	**A. Me.** **(SD)**
Melanomacrophage centers (MMCs)	5,5 [1]	(1.55)	1612.74 (606.28)	14,07 [2]	(8.99)	1388.64 (614.62)	7.39	(4.33)	1514.80 (792.58)

Supplementary figure. Custom library spectra obtained by Raman spectroscopy of target materials. High density polyethylene (HDPE), nylon (PA6), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), textile cellulose (rayon), textile cotton, graphite and silicon.



Supplementary table. Prevalence (P%), mean abundance (MA) and standard deviation (SD) of parasite species of each taxonomic group found in *Mullus barbatus*.

Locality	Barcelona						Blanes		
Year	2007			2018			2018		
Season									
PARASITES	**P%**	**MA**	**SD**	**P%**	**MA**	**SD**	**P%**	**MA**	**SD**
METAZOA									
CNIDARIA									
Myxozoa und.	16.7	-	-	8.6	-	-	17.1	-	-
NEMATODA									
Nematoda und.	56.3	1.98	(2.55)	85.7	3.51	(2.83)	57.1	2.69	(3.10)
Rhabditida									
Hysterothylacium aduncum larva	12.5	0.21	(0.68)	11.4	0.14	(0.43)	8.6	0.09	(0.28)
Hysterothylacium fabri larva	79.2	3.69	(4.25)	91.4	9.09	(8.25)	97.1	22.89	(29.02)
Hysterothylacium sp. larva	37.5	0.81	(1.51)	82.9	2.37	(2.84)	42.9	2.29	(4.02)
Ascarophis sp.	2.1	0.02	(0.14)	5.7	0.06	(0.24)	5.7	0.06	(0.24)
Contracaecum sp. larva	10.4	0.15	(0.55)	17.1	0.29	(0.71)	5.7	0.11	(0.53)
Cucullanus sp.	4.2	0.04	(0.20)	34.3	1.09	(2.25)	14.3	0.31	(1.08)
Dichelyne sp.	-	-	-	11.4	0.17	(0.57)	8.6	0.14	(0.55)
Raphidascaris sp.	-	-	-	2.9	0.03	(0.17)	2.9	0.03	(0.17)
Trichinellida									
Capillaria sp.	12.5	0.13	(0.33)	22.9	0.29	(0.57)	14.3	0.31	(0.90)
Capiloroides sp.	2.1	0.04	(0.29)	11.4	0.14	(0.43)	2.9	0.03	(0.17)
Paracapillaria sp.	4.2	0.06	(0.32)	11.4	0.11	(0.32)	5.7	0.06	(0.24)
PLATYHELMINTHES									
Trematoda									
Digenea und.	31.3	0.58	(1.01)	25.7	0.34	(0.68)	11.4	0.14	(0.43)
Aponurus mulli	10.4	0.19	(0.76)	8.6	0.11	(0.40)	2.9	0.11	(0.68)
Opecoloides furcatus	66.7	1.98	(2.35)	60.0	2.66	(3.79)	65.7	4.77	(6.01)
Paracanthium furcatum	8.3	0.13	(0.49)	11.4	0.11	(0.40)	11.4	0.11	(0.40)
Posornynchus crucibulum	-	-	-	2.9	0.03	(0.17)	-	-	(-)
Protoctrema bacilliobatum	4.2	0.13	(0.73)	5.7	0.14	(0.69)	14.3	0.34	(1.39)
Pseudopecoeloides sp.	6.3	0.06	(0.24)	2.9	0.09	(0.51)	-	-	(-)
Monogenea									
Monogenea und.	2.1	0.02	(0.14)	5.7	0.06	(0.24)	-	-	(-)
Cestoda									
Scolex pleuronectis larva	2.1	0.10	(0.72)	-	-	-	-	-	-
Nybelinia sp.	2.1	0.02	(0.14)	5.7	0.06	(0.24)	5.7	0.17	(0.86)
ARTHROPODA									
Copepoda									
Hatschekia mulli	-	-	-	8.6	0.17	(0.62)	2.9	0.03	(0.17)
Isopoda									
Gnathia sp. (praniza larva)	22.9	0.33	(0.72)	2.9	0.03	(0.17)	-	-	(-)
PROTISTA									
AMOEBOZOA									
Amoeba und.	8.3	-	-	-	-	-	-	-	-
APICOMPLEXA									
Coccidiasina und.	-	-	-	-	-	-	5.7	-	-
CILIOPHORA									
Trichodina sp.	-	-	-	-	-	-	-	-	-
Ciliophora und.	-	-	-	-	-	-	5.7	-	-