Accumulation of PAHs in the tissues and algal symbionts of a common Mediterranean coral: skeletal storage relates to population age structure

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are widespread and harmful environmental pollutants that threaten marine ecosystems. Assessing their level and source is crucial to estimate the potential risks for marine organisms, as PAHs represent an additional threat to organism resilience under ongoing climatic change. Here we applied the QuEChERS extraction method to quantify four PAHs (i.e. acenaphthene, fluorene, fluoranthene, and pyrene) in three biological compartments (i.e. skeleton, tissue, and zooxanthellae symbiotic algae) of adult and old specimens of a scleractinian coral species (*Balanophyllia europaea*) that is widespread throughout the Mediterranean Sea. A higher concentration of all four investigated PAHs was observed in the zooxanthellae, followed by the coral tissue, with lowest concentration in the skeleton, consistently with previous studies on tropical species. In all the three biological compartments, the concentration of low molecular weight PAHs was higher with respect to high-molecular weight PAHs, in agreement with their bioaccumulation capabilities. PAH concentration was unrelated to skeletal age. Observed PAHs were of petrogenic origin, reflecting the pollution sources of the sampling area. By coupling PAH data with population age structure data measured in the field, the amount of PAHs stored in the long term (i.e. up to 20 years) in coral skeletons was quantified and resulted in 53.6 ng m^{-2} of acenaphthene, 69.4 ng m^{-2} of fluorene, 2.7 ng m^{-2} of fluoranthene, and 11.7 ng m^{-2} of pyrene. This estimate provides the basis for further assessments of long-term sequestration of PAHs from the marine environment in the whole Mediterranean, given the widespread distribution of the investigated coral species.

Keywords (max 6)

PAH; bioaccumulation; scleractinian coral; *Balanophyllia europaea*; QuEChERS; Mediterranean Sea

1. Introduction

Coastal marine areas host the most productive, yet threatened, ecosystems in the world (Lazzari et al., 2019). Many interacting natural and anthropogenic stressors, including suspended sediments, nutrients, hypoxia, turbidity, temperature, and pollutants can impair the health and fitness of resident biota (Adams, 2005; Schulte, 2007). Polycyclic aromatic hydrocarbons (PAHs) are a large group of hydrophobic organic compounds whose chemical structure is based on two or more fused benzene rings (Combi et al., 2020). PAHs are among the most hazardous constituents of fuels and oils that threaten marine ecosystems (Rocha and Palma, 2019). Natural sources of PAHs include forest fires, oil seeps, and diagenesis of organic matter and biological processes (Santana et al., 2018; Sun et al., 2018; Thompson et al., 2017). Nevertheless, the main sources of PAHs in the marine environment are related to anthropogenic activities. The main artificial contributors of PAH pollution in aquatic ecosystems result from the incomplete combustion of fossil fuels and organic matter (pyrogenic PAHs) or from ships, industrial discharges, sewage sludge, spills of crude oil and petroleum products (petrogenic PAHs) (Abdel-Shafy and Mansour, 2016; Lawal, 2017; Yang et al., 2019; Combi et al., 2020). PAHs from different sources can enter the marine environment through effluent discharges, surface runoff, marine transport, petroleum spills, and atmospheric deposition (Lin et al., 2013; Santana et al., 2018; Sun et al., 2018; Zhang et al., 2020). Due to their hydrophobic nature, PAHs in the water column are easily adsorbed onto suspended particulate matter and partitioned into sediments (Kim et al., 1999; Neff et al., 2005; Wang et al., 2007; Sun et al., 2018), but can be further remobilized and become bioavailable, thus making benthic organisms (e.g. sessile invertebrates) particularly subjected to PAH bioaccumulation (Frapiccini et al., 2020). Their environmental occurrence raises major ecological concerns, given their high persistency and the serious toxic effects that some PAHs exert on organisms, including teratogenicity, carcinogenicity, and mutagenicity (Frapiccini and Marini, 2015; Wang et al., 2017; Li et al., 2019; Zhang et al., 2020). Based on these features, the United States Environmental Protection Agency has identified 16 PAHs as priority pollutants worldwide (Ko et al., 2014; Nácher-Mestre et al., 2014; Abdel-Shafy and Mansour, 2016; IARC, 2018; Han et al., 2020).

The concentration of PAHs in marine organisms varies in relation to biological aspects (e.g. sex, lipid content, reproduction status), ecological factors (e.g. feeding behavior, trophic levels, habitats), and physicochemical characteristics of the contaminants (El Deeb et al., 2007; Leonards et al., 2008; Rahmanpour et al., 2014; Mashroofeh et al., 2015; Frapiccini et al., 2020). PAH accumulation is also affected by organism biotransformation capacities and by the bioavailability of these compounds, such as PAH concentration in the preys on which organisms feed on (Baumard et al., 1998).

In the Mediterranean Sea, although many studies focus on PAH contamination in benthic organisms such as mollusks (Cocchieri et al., 1990; Ausili et al., 1996; Minier et al., 2006; Perugini et al., 2007; Galgani et al., 2011; León et al., 2013; Mercogliano et al., 2016), crustaceans (Porte and Albaiges, 1993; Perugini et al., 2007; Costa et al., 2016), and fishes (Baumard et al., 1998; Perugini et al., 2007; Solé et al., 2013; Guerranti et al., 2016; Ferrante et al., 2018; Frapiccini et al., 2018, 2020), no study has yet been performed on corals. Corals are benthic organisms in close association with sediments. Therefore, they can be directly exposed to PAHs present in seawater and in resuspended sediments (Yang et al., 2019). Recent investigations report a

wide occurrence of chemical pollutants in corals, suggesting that some characteristics such as growth stage, lipid content, and feeding strategy may play an important role in contaminant accumulation (Yang et al., 2019; Han et al., 2020). Studies on PAH accumulation in corals report the highest concentration in symbiotic algae (i.e. zooxanthellae), followed by the coral tissue, and the lowest one in the skeleton (Ko et al., 2014; Ranjbar Jafarabadi et al., 2018). In general, the amount and types of accumulated PAHs reflect the bioavailable fraction (Thomas and Li, 2000). Nevertheless, coral ability to metabolize these compounds remains unclear (Ranjbar Jafarabadi et al., 2018). This issue is further complicated by the intricated (and only partially disclosed) relationships across all members of the holobiont and the possible roles of microbiota in contaminant accumulation and toxicity towards the coral host (Fragoso ados Santos et al., 2015). Evidence of the adverse effects of PAHs on both coral host and the endosymbiotic zooxanthellae is increasing, with physiological outcomes such as altered gene expression (Woo et al., 2014), metabolic changes (reduced growth rate, increased protein-to-lipid ratios, and shifts from metabolic homeostasis; Guzmán et al., 1991; Downs et al., 2006; Guzmán Martínez et al., 2007), decreased photosynthetic yield, tissue damage and bleaching (Guzmán Martínez et al., 2007), impaired larval development, and settlement inhibition (Overmans et al., 2018; Nordborg et al., 2018).

Corals in the Mediterranean Sea are likely to face the combined effects of climate change and environmental pollution more than in other areas. In fact, the Mediterranean Sea is warming two to three times faster than the global ocean (Vargas-Yáñez et al., 2008), with an increased occurrence of hot extremes (Diffenbaugh et al., 2007). Concomitantly, due to its hydro-geomorphological features (semi-enclosed nature, restricted water exchanges with the Atlantic Ocean), intense coastal urbanization, industrial activity, and heavy shipping, the Mediterranean is influenced by widespread sources of PAHs (Castro-Jiménez et al., 2012). This study focused on *Balanophyllia europaea* (Risso, 1826), a simultaneously hermaphrodite (Goffredo et al., 2002), solitary, and zooxanthellate scleractinian coral living on rocky substratum and endemic to the Mediterranean Sea, where it is widespread (reaching abundances of >100 individuals m^{-2}; Goffredo et al., 2004) both in the Western and the Eastern basin (Ozalp et al., 2019), at depths from 0 to 50 m (Zibrowius, 1980). This species has been deeply investigated for its vulnerability towards ocean warming and acidification in terms of population dynamics (Goffredo et al., 2007, 2008, 2014; Caroselli et al., 2019), mortality rate (Prada et al., 2017), photosynthetic efficiency (Caroselli et al., 2015), reproductive efficiency (Airi et al., 2014), skeletal parameters (Caroselli et al., 2011; Fantazzini et al., 2015; Goffredo et al., 2015), and net calcification rate (Goffredo et al., 2009; Fantazzini et al., 2015). In light of this, PAH contamination of Mediterranean shallow water environments should be considered as an additional threat for coral resilience under ongoing climatic changes.

Acenaphthene, fluorene, fluoranthene and pyrene were chosen among PAH priority pollutants for this study due to their environmental relevance and physicochemical features. Acenaphthene and fluorene have a high bioaccumulation capacity in marine organisms given their propensity for partition in seawater, while fluoranthene and pyrene are more abundant in marine sediments due to their high hydrophobicity (Marini and Frapiccini, 2013). Although these PAHs are not classified as carcinogenic (IARC, 2018), they can induce toxic

reactions in marine organisms (Nácher-Mestre et al., 2014). Mechanisms of toxicity response shown in fishes include trigger of downstream molecular cascades that are involved in the activation of detoxifying enzymes (Cousin and Cachot, 2014). If the toxicants are maintained over a long period, they may saturate the detoxifying enzymes and alter neurochemical/metabolic processes (Little and Finger, 1990), resulting in severe aberrations of the locomotory behavior (e.g. lethargy) (Gonçalves et al., 2008). The aims of this study were to: 1) apply the QuEChERS extraction method to provide a methodological advancement towards a suitable protocol for quantifying PAHs in corals; 2) investigate PAH concentration and origin in three biological compartments (i.e. skeleton, tissue, and zooxanthellae) of *B. europaea*; 3) investigate coral age effects on PAH concentration in *B. europaea* specimens; and 4) quantify the skeletal storage of PAHs in relation to the age structure in a population of *B. europaea*.

2. Materials and Methods

2.1 Coral sampling and study area

On May 31st 2019, thirteen specimens of *B. europaea* were haphazardly collected by scuba divers with a hammer and chisel at a depth of 6 m in Calafuria (43°27' N, 10°21' E, Italy, Ligurian Sea; Fig. 1). The Ligurian Sea is characterized by a narrow continental shelf bordering a deep bottom (~2000 m depth), under the influence of open sea conditions and upwelling currents providing considerable input of nutrients in shallow waters (Cattaneo-Vietti et al., 2010; Casella et al., 2011). Several rivers discharge into the Ligurian Sea, leading to frequent occurrence of seawater high turbidity and nutrient enrichment (Bassano et al., 2000; Attolini et al., 2005). The sampling site of Calafuria is southeast of the port of Livorno, which is one of the largest seaports in the Mediterranean Sea and one of the most polluted sites in Italy (Iannelli et al., 2012). Longshore currents govern a local eastward oriented water circulation, which transports sediments along the coast (Bertolotto et al., 2003). Consequently, polluting agents (i.e. mainly petroleum hydrocarbons and heavy metals resulting from commercial and industrial activities) are dispersed from the Livorno urban and port area to adjacent sites (Bertolotto et al., 2003; Iannelli et al., 2012). The sampling was performed at depths known to have high population densities and where the reproduction, growth rate, and population dynamics of the species are documented (Goffredo et al., 2002, 2004). Upon collection, samples were stored in ice and transferred to the laboratory of the Department of Biological, Geological and Environmental Sciences (Bologna, Italy), where they were stored at -20°C.

2.2 Sample preparation

The length (*L*: maximum axis of the oral disc) of each specimen was measured with calipers (Goffredo et al., 2004, 2007, 2008). The age of each specimen was estimated by applying the length-age relationship previously obtained for this species at the same site and depth (Goffredo et al., 2004). Based on estimated age, samples were categorized in two age classes: Adult ($6 < \text{age (years)} \leq 10$; N = 6) and Old ($10 < \text{age (years)} \leq 14$; N = 7). Coral tissue was removed from the skeleton using an airbrush with filtered artificial seawater (FSW). The extracted tissue was mechanically disrupted using an electrical homogenizer (IKA) for 3 × 10 s. The

homogenate was centrifuged at 5000 g for 5 min at 4°C to separate the zooxanthellae symbiont cells from the coral host tissue. The resulting zooxanthellae pellet was separated from the supernatant and resuspended in 2 ml FSW, centrifuged and resuspended two more times, thus obtaining all the zooxanthellae of the coral suspended in 2 ml (Caroselli et al., 2015). After three centrifugation rounds, the supernatant fractions (host tissue) were pooled. Homogenates containing the coral host tissue or symbiont cells were independently lyophilized. The skeleton of each specimen was treated in a solution of 10% sodium hypochlorite (commercial bleach) for 3 days to completely remove any residue of soft tissue. The skeletons were then washed several times with double distilled water and dried in an oven at 50 °C for 3 days. Each skeleton was then observed under a binocular microscope to remove fragments of substratum and external calcareous deposits produced by epibionts (Caroselli et al., 2011). After these treatments, each skeleton was ground using an agate mortar to obtain a fine and homogeneous powder (Goffredo et al., 2012). The powdered skeleton was weighed with an Ohaus Explorer Pro analytical balance (± 0.0001 g). Lyophilized coral compartments (i.e. tissue and zooxanthellae) and the powdered skeleton were then weighed with a precision balance (± 0.0001 g, Scaltec). The mass of intra-skeletal organic matrix (OM), was estimated as the 2.9% of total skeletal mass, as previously reported for this species sampled at the same site and depth (Reggi et al., 2014). Pollutant concentration in the skeleton compartment (see section 2.3 PAH analysis) was calculated over the mass of OM.

2.3 PAH analysis

The Quick Easy Cheap Effective Rugged and Safe (QuEChERS) method was applied for extraction and purification of selected PAHs (i.e. acenaphthene, fluorene, fluoranthene and pyrene) from skeleton, tissue and zooxanthellae samples of *B. europaea* (Frapiccini et al., 2018). The QuEChERS method is a simple, fast, and valid alternative to conventional extraction methods for multi-residue analysis, as it involves few steps (extraction and clean up), it is low time-consuming and it requires a low amount of solvent (Grimalt and Dehouck, 2016). Originally, it was applied to investigate multiresidue pesticides in agricultural products (Kim et al., 2019). Then, the QuEChERS method was modified and applied to other persistent organic pollutants, including PAHs, from other matrices like fish and seafood (Ramalhosa et al., 2009), but not in non-edible tissue. A comparison between this new method of extraction and a traditional method (accelerated solvent extraction, ASE) was performed by analyzing standard reference material (SRM NIST 1974c). Examined PAHs were extracted with the QuEChERS kit using acetonitrile as the reagent partitioned from the aqueous matrix using anhydrous $MgSO_4$ and NaCl. Samples were purified by a dispersive solid-phase extraction (dSPE) clean up with $MgSO_4$ and primary secondary amine (PSA). The purified extracts were concentrated and recovered with acetonitrile for chemical analysis in UHPLC (Ultimate 3000, Thermo Scientific, Waltham, MA, USA) equipped with a fluorescence (RF2000) detector (Thermo Scientific). A Hypersil Green PAH column (2.1 x 150 mm, 1.8 µm, 120 Å) in a reversed-phase LC with a mobile phase (water:acetonitrile, v/v) gradient elution was used. The flow rate was 0.3 mL min^{-1} at the temperature of 40 °C. Identified PAHs were qualified by their retention time. Analysis of the procedural blanks (N = 6) and the external standard multipoint calibration technique were used to assess quality control. All laboratory blank extract concentrations were

below the limits of quantification (LOQ) for investigated PAHs. Calibration curves were obtained through serial dilutions (from 1:1000 to 1:8000 v/v) from a standard PAH solution (EPA 610 PAH Mix), purchased from Supelco, Bellafonte, PA, USA.

The percentage of recovery was calculated as reported in Table 1. Concentration of PAH compounds was not corrected for surrogate recoveries. Limits of detection (LOD) and LOQ were calculated according to ICH Q2B (ICH, 2005), using the following equations:

LOD = 3.3 *Sa/b*

LOQ = 10 *Sa/b*

where *Sa* is the standard deviation of the intercept of the regression line and *b* is the slope of the calibration curve (Table 1).

2.4 Population life table with PAHs skeletal content

The population age structure ($N_{(t)}$, number of individuals per each age class *t* from 0-20 years, i.e. up to the maximum estimated lifespan), polyp length ($L_{(t)}$), and skeletal mass ($M_{(t)}$) at each age class of *B. europaea* at 6 m depth in Calafuria was derived by Goffredo et al., 2004. The cumulative amount of OM in each age class was calculated by multiplying $N_{(t)}$ by the OM mass in the skeleton for that age class $M_{OM(t)}$. The cumulative amount of each PAH in each age class was calculated by multiplying the mean content of that PAH in all collected skeletal samples (PAH_{SK}) by the cumulative amount of OM in that age class. The total amount of each PAH stored in the skeletons of 1 m^2 of *B. europaea* population at 6 m in Calafuria was obtained by summing up the cumulative content of that PAH in all age classes (Eq. 1).

$$Total\ PAH\ amount = \sum_{t=0}^{t=20}(N_{(t)} \times M_{OM(t)} \times PAH_{SK}) \quad \text{(Eq. 1)}$$

2.5 Statistics

Due to the heteroskedastic dataset, PAH concentration was compared among PAHs, coral biological compartments and age classes with a permutation multivariate analysis of variance (PERMANOVA; Anderson, 2005) based on Euclidean distances using a crossed design with three fixed factors (factor “PAH” with four levels: acenaphthene, fluorene, fluoranthene, pyrene; factor “Compartment” with 3 levels: Skeleton, Tissue, Zooxanthellae; factor “Age class” with 2 levels: Adult, Old) and 999 permutations included the Monte Carlo correction for small sample size. A further PERMANOVA analysis based on Euclidean distances was performed separately for each PAH using a crossed design with two fixed factors (factor “Compartment” with 3 levels: Skeleton, Tissue, Zooxanthellae; factor “Age class” with 2 levels: Adult, Old) and 999 permutations included the Monte Carlo correction for small sample size. PERMANOVA analyses were performed with software Primer 6 (Primer-e Ltd).

3. Results

Acenaphthene, fluorene, fluoranthene, and pyrene concentration was quantified in the skeleton, tissue, and zooxanthellae of adult (N = 6) and old (N = 7) individuals of *B. europaea* collected in the Ligurian Sea (NW Mediterranean Sea; Table 2; Supplementary Table 1, Supplementary Fig. 1). In all biological compartments, both in adult and old individuals, the dominant compound was fluorene, followed by acenaphthene, pyrene and fluoranthene. In addition, fluoranthene had a significantly lower concentration than fluorene, while pyrene generally had an intermediate concentration (Fig. 2; Table 3; Supplementary Table 2). Acenaphthene concentration was clustered with that of fluorene in the skeleton and tissue, while it was clustered with that of pyrene in the tissue and zooxanthellae (Fig. 2; Supplementary Table 2). For all the four PAHs, the concentration in the skeleton was lower than the concentration in the zooxanthellae, while tissue had an intermediate concentration (Fig. 2; Supplementary Table 3). No significant effect of age was observed (Table 3).

For each individual PAH (i.e. analyzed separately from the others), the concentration in the skeleton was significantly lower than the concentration in the zooxanthellae, while the tissue generally had an intermediate concentration (i.e. for fluorene, fluoranthene in old individuals, and pyrene) or the same concentration of the skeleton (i.e. for fluoranthene in adult individuals and acenaphthene; Fig. 3; Tables 4; Supplementary Tables 4 and 5). Age did not show significant effects, with the only exceptions of the concentration of fluoranthene in the tissue and zooxanthellae, that was higher in old individuals than in adults (Fig. 3; Table 4; Supplementary Table 5).

Diagnostic ratio was applied to identify the source of PAHs in the three biological compartments of *B. europaea* samples. The ratio *fluoranthene/(fluoranthene+pyrene)* was used to distinguish between combustion and petroleum sources (Yunker et al., 2002). Most of the samples (95%) exhibited low values of *fluoranthene/(fluoranthene+pyrene)* (<0.4) indicating that PAH contamination originated mainly from petroleum sources (unburned petroleum; Table 5). Only the tissue of two old individuals reflected a combination of petrogenic and pyrolytic contaminations (*fluoranthene/(fluoranthene+pyrene)* > 0.4).

Since no age effect was observed for the concentration of PAHs in the skeleton (Tables 3 and 4; Supplementary Table 5), the amount of each PAH stored in the skeletons of 1 m^2 of *B. europaea* population at 6 m depth in Calafuria was estimated by multiplying the total concentration of each PAH (Table 2) by the amount of OM in the skeleton (Eq. 1), which resulted in 53.6 ng of acenaphthene, 69.4 ng of fluorene, 2.7 ng of fluoranthene, and 11.7 ng of pyrene (Fig. 4; Supplementary Table 6).

4. Discussion

To our knowledge, this is the first study investigating PAHs in a Mediterranean coral species. This is also the first application of the QuEChERS extraction method to quantify PAHs in a coral species. The comparison between this method and the traditional ASE method is shown in Supplementary Table 7. *Balanophyllia europaea* specimens from the Ligurian Sea retained acenaphthene, fluorene, fluoranthene, and pyrene up to about 1 μg g^{-1} dry weight (d.w.), with consistent accumulation pathways between coral skeleton, tissue, and symbiotic zooxanthellae algae. In all three biological compartments, a preferential accumulation of the low

molecular weight PAH compounds fluorene and acenaphthene was observed, in agreement with studies on tropical coral species from the China Sea (Ko et al., 2014; Han et al., 2020) and the Persian Gulf (Ranjbar Jafarabadi et al., 2018). In general, PAHs with 2-3 aromatic rings are more soluble in seawater than PAHs with 4 or more aromatic rings, thus they are more effectively accumulated by organisms (Sverdrup et al., 2002).

PAH concentration in the tissue of *B. europaea* (Table 2) was comparable to that reported for the tissue of the tropical scleractinian *Acropora hyacinthus* from the South China Sea (0.03–0.34 μg g^{-1} dry weight; Yang et al., 2019) and to those recorded in several scleractinian corals from the Persian Gulf (0.16–0.18 μg g^{-1} dry weight; Ranjbar Jafarabadi et al., 2018). Skeletal PAH concentrations reported in this study are expressed in relation to the skeletal fraction of intra-skeletal organic matrix (OM), since this is the lipid storage component and PAH accumulation site in the skeleton. When expressed over the skeletal dry weight, the concentrations of PAHs in *B. europaea* range between 0.0002 and 0.006 μg g^{-1}, which is two orders of magnitude lower than those assessed in skeletons of *Acropora* sp. corals from the Red Sea (0.03–0.3 μg g^{-1} dry weight; El-Sikaily et al., 2003). This may depend on: 1) a lower environmental burden of PAHs in the Ligurian Sea in 2019 than in the Egyptian Red Sea in 1999 (El-Sikaily et al., 2003), 2) a higher skeletal storage capacity of *Acropora* sp. with respect to *B. europaea*, likely related to a species-specific difference in OM and/or lipid content in the skeleton; 3) different metabolic capacities of the two species towards PAHs, or 4) a combination of these factors. The literature lacks studies on species-specific or location-specific differences in coral skeletal PAH concentration, highlighting the need to increase the basic research effort on PAH contamination in corals and the related physiological outcomes.

The accumulation pattern of all investigated PAHs in *B. europaea* was: zooxanthellae > coral tissue > coral skeleton, in agreement with studies on other coral species from different locations (Ko et al., 2014; Ranjbar Jafarabadi et al., 2018), suggesting that this could be a common pattern. The distinct organic pollutant accumulation capacity in corals tissues (soft and skeleton) and zooxanthellae may be related to the lipid content, since the bioaccumulation of hydrophobic compounds is affected by the amount and relative composition of lipids within biological compartments (Kennedy et al., 1992; Readman et al., 1996; Samorì et al., 2017). In this light, performing similar investigations on non-zooxanthellate corals, where symbiotic algae-associated lipids are not present, may give relevant insights on the effect of symbiosis in PAH accumulation and metabolic pathways in corals.

The petrogenic origin of detected PAHs reflects the impact of petroleum contamination at the sampling site (Bertolotto et al., 2003; Iannelli et al., 2012). Since all biological compartments had a similar PAH origin, the following pathway of accumulation may be hypothesized: PAHs are first absorbed by zooxanthellae and translocated through lipid storage to the coral soft tissue (Krueger et al., 2018; Hambleton et al. 2019; Radice et al. 2019) and then to the OM (Reggi et al., 2016; Samorì et al., 2017), where lipids are present as free fatty acids, phospholipids, sterols, ceramids, and sterol esters (Farre et al., 2010), likely serving as $CaCO_3$ nucleation sites (Isa and Okazaki, 1987). Furthermore, corals use lipid vesicles for ion transport to the sites of mineralization, after which lipids are incorporated into the growing skeleton (e.g. Samorì et al., 2017). A further source of PAH contamination in corals may be predation on zooplankton, a feeding strategy that is

present in all coral species, with different degrees of importance depending on the heterotrophic/autotrophic ratio shown by zooxanthellate species. Zooplankton accumulate PAHs (Almeda et al., 2013; Ziyaadini et al., 2016; Hsieh et al., 2019) and is a relevant source of organic pollutants in low trophic level feeding organisms (Wan et al., 2007; Alekseenko et al., 2018). Given their high solubility, PAHs may enter coral tissues also through coral mucus (Wild et al., 2004). Furthermore, contaminants adsorbed onto the particulate matter trapped on the surface of coral mucus may enter the coral (Zhang et al., 2019; Han et al., 2020). PAHs accumulated by the zooxanthellae, zooplankton, and coral mucus may further circulate between biological compartments depending on physiological processes (e.g. skeletal biomineralization). Biotransformation of organic pollutants may also play a determinant role in PAH bioaccumulation. In this regard, it is worth noting that data on detoxification/biotransformation mechanisms in corals are very scarce. Considering the general assumption of relatively slow coral growth rate (Goffredo et al., 2004), PAH levels assessed in *B. europaea* in this study may result from the effective uptake routes depicted above, and from a low biotransformation efficiency for PAHs in live tissues, which are further affected by algae contribution to uptake and/or degradative processes (Gust et al., 2014). The skeleton may be the final repository of both parental and metabolic compounds.

Coupling PAH concentration data with population structure of *B. europaea* at Calafuria (Goffredo et al., 2004) allowed to estimate the amount of PAHs stored in the OM, and thus to evaluate the capacity of natural coral populations to sequester and immobilize PAHs for a relatively long time (20 years = maximum estimated longevity of corals in the investigated population; 4 years = turnover time; i.e. average age of individuals in the population; see Goffredo et al., 2004 for parameter estimation). The content of PAHs in the population reached a maximum in individuals of 6 years of age. Younger individuals are more represented in the population, but their PAH content is low, given their small size and skeletal mass. Individuals older than 6 years are so rare that their contribution to the overall population PAH content is lower. PAHs trapped in the skeleton become unavailable for animal metabolic mechanisms and thus are stored in a non-biologically active form until coral death and skeletal dissolution occurs. Under the scenarios of projected ocean acidification trends (IPCC, 2019), which is expected to speed-up the dissolution of shallow water carbonates, including coral skeletons, this process is particularly relevant, and its fine-scale investigation is urgent. In this context, since physiological traits related to growth and biomineralization vary widely among populations of *B. europaea* located throughout the latitudinal extension of Italian coasts (>1000 km; Goffredo et al., 2008), applying the experimental setup employed in this study across this gradient (currently underway) will likely provide an accurate and wide range estimation of how coral biomineralization buffers PAH contamination in coastal environments. To improve the detection of possible age effects on PAH concentration that were not identified in the present study, the sampling range should be expanded to include younger individuals (<6 years), which are sexually inactive (<3-4 years: Goffredo et al. 2004) and whose different physiology may alter the concentration of PAHs in their biological compartments.

In conclusion, this study showed that the Mediterranean coral *B. europaea* accumulates acenaphthene, fluorene, fluoranthene and pyrene likely as a result of its mixotrophic strategy, comprising both zooplankton

predation and macromolecules (in particular lipids) acquisition from the symbiotic partnership with the zooxanthellae algae. Low molecular weight PAHs were preferentially accumulated compared to high molecular weight PAHs, with higher concentrations in the symbiotic algae, followed by the host tissue, and finally in the skeleton. This trend is common to other coral species analyzed outside the Mediterranean Sea. Detected PAHs were of petrogenic origin, reflecting pollution sources of the sampling area. PAHs were effectively stored in the skeletons of *B. europaea*, likely due to their hydrophobicity and interaction with lipids. Skeletal lipids contribute to the formation and function of the OM, the non-mineral fraction composed of a framework of macromolecules (besides lipids, it includes proteins, glycoproteins, and polysaccharides) that regulates biomineral deposition and skeletal developmental patterns (Goffredo et al., 2011). Therefore, the possible PAH partitioning in and interaction with OM macromolecules may represent a threat for coral biomineralization, as already reported for vertebrate bone mineralization (Duan et al., 2018; Zanaty et al., 2020). Besides evaluating potential detrimental effects, the quantification of PAHs stored in coral skeleton reported in this study provides the basis for further assessments of long-term sequestration of PAHs from the environment in the whole Mediterranean, given the widespread distribution of the target coral species.

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Figure legends

Fig. 1 Location where corals were collected. (a) Map of Calafuria (43°27' N, 10°21' E, Italy, Ligurian Sea). (b) Specimens of the common and abundant coral *B. europaea* on a rock at 6 m in Calafuria.

Fig. 2 Concentration (µg g^{-1} of dry weight) of PAHs in the three biological compartments of *B. europaea*. (a) Boxplots represent median, upper and lower quartiles (N = 13) of PAH concentration in coral skeleton, tissue, and zooxanthellae. Different letters indicate significant differences in the concentration of each PAH between biological compartments ($P < 0.05$; PERMANOVA pairwise comparisons t-tests; 999 permutations). (b) Within each biological compartment, triangular matrices report differences between pairs of PAHs (**$P < 0.01$, *$P < 0.05$; PERMANOVA pairwise comparisons t-tests; 999 permutations).

Fig. 3 Concentration (µg g^{-1} of dry weight) of individual PAHs (acenaphthene, fluorene, fluoranthene and pyrene) according to age classes in the three biological compartments of *B. europaea.* Boxplots represent median, upper and lower quartiles of PAH concentrations in coral skeleton, tissue and zooxanthellae in adult (N = 6) and old individuals (N = 7). Different letters indicate significant differences in the concentration of each PAH between biological compartments and/or age classes ($P < 0.05$; PERMANOVA pairwise comparisons t-tests; 999 permutations).

Fig. 4 PAH storage in the skeletons of *B. europaea* in 1 m^2 of population at 6 m depth in Calafuria (Italy, Ligurian Sea), according to population age structure. (a) Distribution of the number of individuals (solid line), and OM mass (dotted line) with coral age. (b) PAH mass stored in the skeleton (blue = fluorene, pink = acenaphthene, yellow = pyrene, red = fluoranthene) over the age of *B. europaea* specimens.