Uptake of aquaculture-related dissolved organic pollutants by marine sponges: Kinetics and mechanistic insights from a laboratory study

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Abstract

Besides the release of organic matter from uneaten feed and fish excreta, a considerable amount of deleterious chemicals may also end up into the marine environment from intensive aquaculture. A fraction of these pollutants remains freely dissolved and pose a threat to marine life due to increased bioavailability. Given the filter-feeding ability of sponges, we investigated the capacity of four ubiquitous Mediterranean species (*Agelas oroides*, *Axinella cannabina*, *Chondrosia reniformis* and *Sarcotragus foetidus*) in removing aquaculture-related dissolved organic pollutants. These included individual chemicals belonging to antibiotics (i.e., oxytetracycline), antifouling biocides (i.e., diuron and Irgarol 1051) and polycyclic aromatic hydrocarbons (i.e., 2,6-dimethylnaphthalene, phenanthrene). The uptake of pollutants was assessed in vitro by exposing small sponge explants to each chemical for a period of 8 h. Additional "cleanup" experiments were performed for complex mixtures mimicking the dissolved organic material encountered in fish farms, such as filtrates of fish feed and excreta. All sponges exhibited a pronounced preference for lipophilic pollutants and a strong positive correlation was revealed between clearance rate and substrate hydrophobicity. Our best filter-feeder (i.e., *A. oroides*) was able to clear 10.0 ± 1.3 mL of seawater per hour and per gram of sponge, when exposed to 2,6-dimethylnaphthalene. Active pumping was found to be the predominant mechanism dictating the assimilation of dissolved pollutants in all sponge species, as it was 3-10 times faster than pollutants' passive adsorption on sponges' pinacoderm. Additionally, the uptaken pollutants were shown to be strongly retained by sponges and they were hardly released back to seawater as a result of desorption or sponge excretory mechanisms. Our study corroborates that sponges are highly efficient in uptaking dissolved organic compounds and it offers new insights into the kinetics and mechanisms ruling this process.

Keywords: Aquaculture wastes, organic pollutants, Dissolved Organic Matter (DOM), Mediterranean sponges, bioremediation, uptake kinetics

1. Introduction

Aquaculture constitutes the driving force behind the growth in global fish production. Given the rising urgency for food sustainability, as well as the current dietary trends towards a better health, the specific economic sector has experienced great demand over the last few decades and will continue to expand, reaching 109 million tonnes in 2030 (FAO, 2020). However, intensification of fish farming has raised a series of environmental concerns. These are mainly associated with the release of large quantities of organic waste into the marine environment, predominantly generated from the dispersion of uneaten feed, fish faeces and soluble excretory products (Rosa et al., 2020). To enhance productivity and growth, a wide range of chemicals are used in fish farms, a considerable amount of which ends up in the water column with sound threats engendered for the marine ecosystem. Such compounds include antibiotics, which are administrated to farmed species to control disease, pesticides to control parasites and algae, as well as antifouling agents and booster biocides to prevent the development of epibionts (i.e., marine biofouling) on the submerged infrastructures (Tornero and Hanke, 2016; Yebra et al., 2004). What is more, increased boating activity in proximity to fish farm facilities, can potentially pose an additional source of organic effluent through the release of petroleum-related combustion byproducts (Nasher et al., 2013).

Sponges (Phylum: Porifera) have recently been viewed as promising bioremediators in integrated aquaculture systems (Fu et al., 2006; Gökalp et al., 2021; Longo et al., 2022; Milanese et al., 2003; Pronzato et al., 1998). The innate capability of these sessile invertebrates to filter large volumes of water, by retaining efficiently different types of particulate organic matter (POM) including bacteria (Claus et al., 1967; Longo et al., 2010; Maldonado et al., 2010; Stabili et al., 2006, 2008; Wehrl et al., 2007; Zhang et al., 2010), phytoplankton (Frost, 1978; Osinga et al., 2001; Riisgård et al., 1993; Varamogianni-Mamatsi et al., 2021) and even synthetic latex microspheres (Turon et al., 1997), has been well-documented. Many studies have provided further insights into the mechanisms by which sponges are able to capture suspended particles (Maldonado et al., 2010; Reiswig, 1971; Van Well, 1949; Weissenfels, 1992).

The ability of sponges to feed on dissolved organic matter (DOM) has also been postulated (de Goeij et al., 2013), but the few existing in vitro attempts to assess this capacity are limited to conventional organic nutrients, such as amino acids (e.g., glycine; Stephens and Schinske, 1961) and sugars (e.g., fructose;

Camacho et al., 2006). Additional laboratory studies examining small particles, such as 0.1 μm nanospheres (Leys and Eerkes-Medrano, 2006) and viruses (Hadas et al., 2006), have been used in support to the sponges' capacity for DOM feeding (considering that these substrates pass through 0.2-μm filter and they are technically included in the dissolved organic pool). Furthermore, Ribes et al. (1999) measured the in situ grazing rates of the temperate sponge *Dysidea avara* over an annual cycle, only to discover that the specific species was more a producer, rather than a consumer of dissolved organic carbon (DOC). It was Yahel et al. (2003) who revealed that most of the carbon gained by the reef sponge *Theonella swinhoei* was in dissolved (<90%) rather than in particulate form, highlighting the importance of DOC in metazoan nutrition and the role of these animals in carbon cycling. In the same context, de Goeij et al. (2008) reported the highest ever DOC removal rates for three encrusting reef sponges under in situ conditions.

Although enlightening, the above findings do not focus on the bioremediation capability of these filter-feeding animals to reduce marine pollution resulting from aquaculture operation. It was Pronzato and his co-workers (1998, 1999) who first attempted to cultivate the Mediterranean bath sponges *Spongia officinalis* and *Hippospongia communis* near floating-cage fish farms in Kalymnos Island for the mitigation of the aquaculture organic wastes. However, their study was only limited to growth and survival rates of sponges, rather than the dynamics of the organic matter uptake. Furthermore, Fu et al. (2007) conceptualized the marine sponge *Hymeniacidon perlevis* as a potential bioremediator in integrated aquaculture ecosystems and they investigated the capacity of this species to remove organic detritus and excretions from the fish *Fugu rubripes*. Though, it is worth stressing that their study was targeted only to Total Organic Carbon (TOC) removal. Later on, the same sponge was proved to be able to remove the organochlorine pesticide lindane from seawater under laboratory conditions (Aresta et al., 2015). Other studies have further indicated the bioaccumulation of organic pollutants in marine sponges, including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (Gentric et al., 2016; Perez et al., 2003).

While the uptake of dissolved organic compounds by sponges has been previously investigated in a handful of studies, the kinetics of this process were scrutinized only in one of them (e.g., fructose; Camacho et al., 2006). Relevant research on the uptake kinetics of organic pollutants is completely absent. In the present study, a series of in vitro experiments was performed using four ubiquitous Mediterranean sponge species

and their uptake kinetics against aquaculture-related pollutants, as well as complex organic mixtures, were assessed. We further aimed to clarify some key mechanistic aspects related to DOC uptake by sponges. In this context, we investigated passive adsorption of pollutants onto sponge biosurface and we assessed the contribution of this process to the overall uptake rate. We also examined whether the pollutants uptaken by sponges can be released back to seawater (i.e., via desorption or sponges excretory mechanisms). To the best of our knowledge, this is the first study to examine the uptake of various organic pollutants by several sponge species and provide comprehensive kinetic information about this process, accompanied by valuable mechanistic insights.

2. Materials and methods

2.1. Study sponge species

The filtering activity towards dissolved organic compounds was examined over four demosponge species that are commonly distributed in high abundances along eastern Mediterranean habitats (Voultsiadou, 2005), namely: (a) *Agelas oroides*, (b) *Axinella cannabina*, (c) *Chondrosia reniformis*, and (d) *Sarcotragus foetidus*. The selection, sampling, transportation, acclimation and maintenance of the particular sponge specimens has been thoroughly described in a previous study (Varamogianni-Mamatsi et al., 2021). The main characteristics of those species are only briefly discussed below.

Agelas oroides (Schmidt, 1864) is a massive, variably lobate-digitate, vivid orange-colour demosponge, generally 5–25 cm in height and common in 2–40 m water depth. It preferably dwells in habitats with low light intensity (Ferretti et al., 2009; Idan et al., 2020). The erect, branching *Axinella cannabina* (Esper, 1794) mainly occurs along sciaphilous hard-bottom assemblages of the Mediterranean basin (Idan et al., 2018). The particular metazoan owns many short inner canals and can reach 55 cm in height (Gerovasileiou et al., 2015; Koukouras et al., 1996). The demosponge *Chondrosia reniformis* (Nardo, 1847) lacks typical skeletal structures (i.e., siliceous spicules or spongin fibers) and is particularly known for its unusual collagenous texture and regenerative properties, which have attracted great biotechnological interest (Nickel and Brümmer, 2003). Shaded environments of the littoral zone (0-50 m) commonly host this species (Wilkinson and Vacelet, 1979). *Sarcotragus foetidus* (Schmidt, 1862) is a variably dark-colour, rather common

Mediterranean keratose demosponge, which approximates an irregularly globular to massive growth form, generally reaching 1 m in diameter and 50 cm in height. It is commonly found in shallow habitats exposed to light, but also in darker zones up to 400 m in depth (Manconi et al., 2013).

2.2. Substrates tested for sponge uptake

2.2.1. Chemical pollutants

The organic constituents selected for the "cleanup" experiments were covering different levels of lipophilicity and represented typical pollutants of various aquaculture settings. These are presented in **Table 1** along with some information about their use/origin and their octanol-water partition coefficients.

2.2.2. DOM from biological extracts

The assimilation capacity of sponges was further examined against two complex mixtures of dissolved organic compounds, which mimicked aquaculture wastes and resulted from the filtration of water-solubilized fish feed and fish excreta. In the first case, solid feed pellets were mixed with ultrapure water and subsequently homogenized at ambient temperature using a conventional mixer. The solution was centrifuged (9500 g for 15 min) and the supernatant was then passed through a 0.45 μm polyethersulfone (PES) filter to remove any remaining submicron-sized particles. In the second case, fresh, crude samples of faeces were collected from fish breeding tanks of *Sparus aurata* and subsequently, homogenized at ambient temperature using a conventional mixer. The mixture was further centrifuged (7000 g for 15 min) and dissolved organic fraction was obtained through filtration of the supernatant (a 0.45 μm pore size). Stock solutions of the two DOC substrates were stored at 4 ^{o}C until their use in sponge "cleanup" experiments.

2.3. Sponge-based experiments

2.3.1. Uptake of chemical pollutants and DOM by sponges

The experimental protocol followed for investigating the uptake of typical aquaculture pollutants was based on the methodology proposed by Varamogianni-Mamatsi et al. (2021) with a few modifications, depending on the applied substrate. In detail, the lab-scale experiments were performed in 2-L glass jars filled with 1 L of natural seawater (NSW) collected from sponge storage tanks and supplemented with the pollutant of interest. To ensure complete and fast dissolution of organic pollutants, crystals of each compound (~2 mg) were initially mixed with a small volume of organic solvent (e.g., 400 μL methanol) and then added in a carboy

containing 20 L of NSW to reach a final concentration of ~100 μg L^{-1}. Jars were filled with 1-L aliquots of the solution and sponges were subsequently immersed in. For each chemical reagent tested, a total of twelve jars were prepared with explants of the four sponges (three replicates for each sponge species), while another three jars containing NSW and substrate (without sponge) served as controls. Over the experimental time course, aeration was applied by an air pump to achieve adequate water mixing and maximize oxygen levels. For the more volatile substrates, such as phenanthrene and 2,6-dimethylnaphthalene (2,6-DMN), gentle rod-stirring was applied instead of aeration to minimize evaporation losses. Water samples of 1 mL were collected at regular time intervals (0, 1, 3, 5, 7, 8 h) and subjected to HPLC analysis. Regarding the DOM derived from fish feed and excreta, aliquots of their stock solution were diluted in NSW to reach a final concentration of 20-30 mg L^{-1} before sponge experiments. Water samples of 8 mL were collected from each jar at the same time intervals as described above and subjected to organic carbon analysis. In addition, the wet weight of each sponge explant was measured at the nearest 0.1 g prior and after the experiments to verify weight stability and the average value was used in subsequent calculations.

2.3.2. Assessment of pollutants desorption/release from sponges to seawater

Best-performing explants (i.e., one specimen of each sponge species) were immersed in seawater containing ~100 μg L^{-1} of phenanthrene and exposed to the chemical for 16 h. The wet weight of the specific explants was 151 g for *A. oroides*, 23 g for *A. cannabina*, 67 g for *C. reniformis* and 70 g for *S. foetidus*. Water samples collected before and after exposure were analyzed by HPLC to quantify the amount of phenanthrene uptaken by sponges. Subsequently, the explants were transferred in jars with fresh, pollutant-free NSW and 1-mL aliquots were collected at the same time intervals as previously described. The samples were analyzed by HPLC to assess the release of phenanthrene that may occur due to desorption or sponge excretory processes.

2.3.3. Assessment of passive adsorption of pollutants onto sponge biosurfaces

Best-performing explants of each sponge species were sacrificed by overnight freezing at -20 ^{o}C. Dead sponges lacking filtration/uptake activity were subsequently immersed in NSW containing ~100 μg L^{-1} of phenanthrene. Water samples were collected and analyzed as described above to track phenanthrene removal due to passive adsorption on sponge biosurfaces.

2.4. Sample Analysis

2.4.1. Chemical pollutants

Concentrations of the contaminants were measured in seawater over the course of sponge-uptake experiments using an Agilent 1260 Infinity Binary Pump HPLC system coupled with a UV-Vis diode array detector (Agilent Technologies). Chromatographic separation of analytes was achieved with a Zorbax Eclipse Plus column (EC-C18, 2.1 × 50 mm, Agilent Technologies), by setting injection volume to 20 μL, temperature at 35 ^{o}C and flow rate of mobile phase at 0.5 mL min^{-1}. Mobile phase A was a mixture of water:methanol (95:5 v/v), while phase B was pure methanol. Mobile phase additives were not used with the exception of oxytetracycline (OTC) analysis, where formic acid was added in phase B (0.1% final concentration) to enhance chromatographic performance. A gradient elution program with a run time of 12 min was used as follows: 0 min: 10% B, 5 min: 100% B, 9 min: 100% B, 9.5 min: 10% B, 12 min: 10% B. The detection of pollutants was achieved spectrophotometrically at wavelengths of maximum absorbance (with 8 nm bandwidth), as follows: OTC, 356 nm; diuron, 250 nm; Irgarol 1051, 225 nm; 2,6-DMN, 224 nm; phenanthrene, 254 nm.

2.4.2. DOM

For the quantification of DOM levels over the course of sponge "cleanup" experiments with complex organic mixtures, seawater samples were subjected to a Total Organic Carbon Analyzer (Shimadzu TOC-$L_{CSN/CSH}$, Japan) equipped with a high-salt combustion tube kit. The method of Non-Purgeable Organic Carbon (NPOC) was employed following manufacturers' default settings. The analyses were performed by setting an injection volume of 50 μL, three replicates per sample and maximum coefficient of variation at 1.5%. A calibration curve was generated using standard solutions of potassium hydrogen phthalate. All NPOC measurements were subjected to blank subtraction to adjust for the minimal background levels of DOC in NSW.

2.5. Data Analysis

2.5.1. Uptake kinetics and clearance rate (*c*)

Coughlan (1969) and, later on, Ferguson (1980) proposed the following exponential decay equation of the DOM in the NSW to describe the uptake of dissolved organic compounds by filter-feeders:

$$C_t = C_0 \times \mathrm{e}^{-c \cdot \mathrm{w} \cdot \mathrm{t/V}} \quad (1)$$

where C_0 and C_t are the concentrations of each pollutant in the treatment jar at the beginning of the

experiment and at time *t*, respectively, *c* is the clearance rate (mL h^{-1} g^{-1}), V represents the volume of NSW (i.e., 1000 mL) in the jars, t is the time (h) and w is the wet weight of sponge (g).

Apart from the uptake driven by sponge's filtering activity, other processes may also contribute to the overall loss of chemical compounds in NSW, such as photooxidation (ECHA, 2018; Ogura, 1972; Sakkas et al., 2002; XiaoWu and Shao, 2017), adsorption on plastic materials (e.g., pipete tips/glass surfaces) (Buma et al., 2009), or volatilization (ECHA, 2018). Pollutants removal caused by those external factors was assumed to follow first-order kinetics, as shown in the equation below:

$$C_t = C_0 \times \mathrm{e}^{-k \cdot \mathrm{t}} \quad (2)$$

where *k* (h^{-1}) is the corresponding pollutant kinetic constant.

The depletion of organic substrates in our study systems was assumed to result from both sponge's filtering activity and external factors. Thus, the overall removal can be described by the following model, which derives from the sum of the **Eq. (1)** and **Eq. (2)**:

$$C_t = C_0 \times \mathrm{e}^{-\left(\frac{c \cdot \mathrm{w}}{\mathrm{V}} + k\right) \cdot \mathrm{t}} = C_0 \times \mathrm{e}^{-k_{total} \cdot \mathrm{t}} \quad (3)$$

where k_{total} (h^{-1}) is the kinetic constant governing the overall substrate removal over the experimental time course.

For each sponge system, k_{total} kinetic constants were obtained by fitting **Eq. (3)** to the experimental C-t data. The respective *k* values describing pollutants removal by external factors were also derived after fitting **Eq. (2)** to experimental data from control systems (experiments without sponges). Finally, the clearance rates (*c*) corresponding to sponge's filtering activity alone were calculated from the following equation:

$$c = \frac{\mathrm{V}}{\mathrm{w}}(k_{total} - k) \quad (4)$$

2.5.2. Retention rate (*r*)

According to Wehrl et al. (2007), retention rate is the term used to describe the quantity of a substrate that is uptaken by sponges, normalized to their wet weight (g) and time of exposure (minutes):

$$r = \frac{1-\left(10^{y60}\right)}{\mathrm{w}} C_0 \mathrm{V} \quad (5)$$

where *y* is the slope of the semi-logarithmic graph of C_t versus t for the linear time interval, multiplied by 60 to give retention rates per hour.

2.5.3. Retention Efficiency (RE)

To estimate the ability of sponges to remove dissolved organic substrates from NSW in a percentage scale, we used the term Retention Efficiency (RE), as proposed by Stabili et al. (2006):

$$\mathrm{RE} = \left(\frac{C_0 - C_t}{C_0}\right) \times 100\% \quad (6)$$

2.6. Statistical Analysis

Analysis of variance (ANOVA) was used to test the differences between different experimental groups. Statistically significant differences were identified by setting the level of significance at $p<0.05$.

3. Results and discussion

3.1. Kinetics of organic pollutants removal by marine sponges

A total of 75 laboratory experiments was performed in order to investigate the removal of five organic pollutants from seawater using four different species of marine sponges. The experiments were conducted in five independent sets, where three explants of each sponge species were immersed in seawater containing a pollutant of interest, and three extra jars were sponge-free (i.e., containing only the pollutant solution) to serve as control systems. Water samples were analyzed at regular intervals to determine the change in pollutants level over time. In all cases, the initial concentration of each compound was set to 100 μg L^{-1}. It is worth stressing that toxicity effects on sponges were not visually observed during their exposure to pollutants. **Fig. 1A–E** shows the concentration-time profiles of OTC, diuron, Irgarol 1051, 2,6-DMN and phenanthrene over an 8-h treatment with *Agelas oroides*, *Axinella cannabina*, *Chondrosia reniformis* and *Sarcotragus foetidus*, as well as under the absence of any sponge (controls). Plotted data points represent the average values of three biological replicates (three sponge explants), while the error bars indicate standard deviation. The concentration of all chemicals showed a time-dependent decrease in the presence of sponges, the actual magnitude of which varied among the different species. More specifically, 2,6-DMN and phenanthrene exhibited the most striking decrease, particularly when these compounds were subjected to *A. oroides*, while the change in OTC levels was hardly noticeable after 8-h exposure to any sponge species. An intermediate behavior was evident in the kinetic profiles of diuron and Irgarol 1051.

A progressive but less profound decrease of concentration was also observed in the control experiments of

Irgarol 1051 and 2,6-DMN, as well as for phenanthrene to much lesser extent, which complicated the evaluation of sponges' efficiency on pollutants removal. This finding implied that, in addition to sponge uptake, the decline of chemical substrates was also modulated by other processes, such as degradation, evaporation, adsorption or a combination of them. Indeed, Irgarol 1051 has been shown to be susceptible to photodegradation in natural waters (Sakkas et al., 2002), while adsorption on plastic materials (e.g., pipete tips) or glass surfaces has been also suggested to cause quick losses of this compound (Buma et al., 2009). On the other hand, 2,6-DMN was by far the most volatile compound among those tested (followed by phenanthrene) and dimethylnaphthalenes have been reported to undergo rapid volatilization and/or photodegradation when present in aqueous systems (ECHA, 2018). Regardless of the process dictating the results in control experiments, the concentration-time profiles of all five pollutants in the absence of sponges were highly reproducible across replicates (average RSD of analyte concentrations was less than 10%) and well described by first-order kinetics (R^2: 0.91-0.95).

The data from sponge experiments were also fitted to first-order kinetics (**Eq. (3)**) and the influence of controls was subtracted to derive models describing the net effect of sponges on pollutants removal (**Eq. (1)**). The latter were used to simulate the decrease of pollutants in seawater solely driven by the filtration activity of sponges (**Fig. S1**). Based on this approach, all four types of sponges retained only 2-8% of OTC within 8 h, while the respective retention efficiency for diuron varied from 10 to 35%. A high retention efficiency of Irgarol 1051 was found for *S. foetidus* (79%), while moderate values were observed for the rest of the sponges (18-45%). With regard to 2,6-DMN and phenanthrene, *A. cannabina*, *C. reniformis* and *S. foetidus* demonstrated a similar behavior and removed 57-68% and 56-70% of those two pollutants, respectively. A more impressive effect was evidenced for *A. oroides*, which caused complete removal of 2,6-DMN and phenanthrene within ~4 h.

Previous studies dealing with the uptake of individual organic compounds by sponges are very scarce. Aresta et al. (2015) exposed *H. perlevis* to the organochlorine pesticide lindane and they observed a 50% decrease within 2 days. Given the differences in sponge species and substrates, a detailed comparison with our results is of limited value, but overall lindane removal seems to follow slower kinetics. Interestingly, lindane-degrading bacteria were isolated from *H. perlevis* and they were suggested to contribute to the removal of

this pesticide. Another study from early '60s examined amino acids uptake by 35 marine invertebrates including sponges (Stephens and Schinske, 1961). By performing laboratory experiments with *Cliona celata* and *Microciona prolifera*, a decrease in glycine levels by 38% and 90% was observed respectively, within ~20 hours. Since amino acids are essential nutrients readily assimilated by all types of organisms, seawater was amended with antibiotics to minimize glycine uptake by microorganisms. Still, an effect from microbial growth cannot be ruled out in that study, as sponges and other seawater organisms have been frequently reported to host antibiotic-resistant bacteria (Laport et al., 2016; Phelan et al., 2011; Smaldone et al., 2014). In a similar investigation, faster kinetics were observed for fructose uptake by *Crambe crambe*, as the concentration of the specific substrate decreased by ~42% within 6 hours (Camacho et al., 2006). Though, sponge excretions were suspected to interfere with fructose measurements, which were implemented with a universal carbon analyzer.

Clearance and retention rates are more appropriate measures for assessing the kinetics of pollutants removal by sponges, because the mass of the filtering organism is taken into account. The specific parameters were calculated using the wet weight of each sponge explant, while the average values were 81.5 ± 31.4 g, 32.3 ± 7.0 g, 67.7 ± 23.5 g and 86.1 ± 10.2 g for *A. oroides*, *A. cannabina*, *C. reniformis* and *S. foetidus*, respectively. Highly varying results were obtained for both clearance (0.03 to 10.0 mL h^{-1} g^{-1}) and retention rate (4 to 383 $ng_{chemical}$ h^{-1} g^{-1}) across sponges and chemical substrates (**Table 2**). The one-way ANOVA test indicated significant differences between the clearance rates of the four sponge species for each pollutant investigated, while the same was also true for retention rates (**Table S1**). These differences resulted primarily due to the significantly higher values that *A. oroides* and *S. foetidus* presented in comparison to the other sponge species. More specifically, the clearance and retention rates of *A. oroides* for 2,6-DMN, phenanthrene and diuron were roughly 2-6 times higher compared to the other sponges, while *S. foetidus* exhibited 2-4 times higher rates for Irgarol 1051 and oxytetracycline.

With the exception of *S. foetidus*, ANOVA analysis also revealed significant differences among the five organic pollutants for each individual sponge investigated (**Table S2**). This outcome was largely driven by the significantly higher "cleanup" parameters obtained for phenanthrene and 2,6-DMN. Both of them demonstrated up to 284 times higher clearance rates and up to 76 times higher retention rates compared to

all other compounds. By scrutinizing these results, a positive association between the "cleanup" parameters of the pollutants and their lipophilicity was suspected. By preparing log-log plot of clearance rates of the five pollutants versus their octanol-water partition coefficients (K_{OW}) (**Fig. S2**), a linear relationship was observed for each one of the four sponge species (R^2: 0.84-0.95, $p<0.05$). The equation describing the relationship of those two terms was Log(*c*)=0.4017*Log(K_{OW})-1.0959 for *A. oroides*, Log(*c*)=0.3168*Log(K_{OW})-1.0592 for *A. cannabina*, Log(*c*)=0.3508*Log(K_{OW})-1.2443 for *C. reniformis* and Log(*c*)=0.2224*Log(K_{OW})-0.7709 for *S. foetidus*. With the exteption of *S. foetidus*, all other sponge species resulted in equations with almost identical slopes. Strong positive correlations with K_{OW}, accompagnied by similar curve slopes, were also evident in the respective plots of retention rates (**Fig. S3** and **Table S3**). Despite the aforementioned dissimilarities in the magnitude of clearance/retention rates among sponges (**Table 2**), the consistency in curve slopes implys that the mechanism governing the kinetics of organic pollutants uptake by the different sponges is of similar nature and K_{OW}-dependent.

Bioacummulation of pollutants in marine biota, representing the result of dynamic equilibrium between the uptake (both waterborne and dietary) and elimination processes taking place, has been extensively studied. In the case of persistent organic pollutants, which are hardly metabolized or degraded, bioaccumulation practicaly reflects the dynamics of pollutants uptake and this has been widely documented to be highly correlated with pollutants hydrophobicity in various types of aquatic organisms (Ding et al., 2019; Oliver and Niimi, 1988). Notably, strong positive correlations with Log K_{OW} have been reported for the bioconcentration of organic pollutants (i.e. waterborne-only uptake) in filter-feeders, such as mussels (Endicott et al., 1998; Geyer et al., 1991). In relation to those findings, our results suggest that the lipophilicity of organic pollutants has a dominant effect not only on the dynamics but also on the kinetics of their uptake by filter-feeder organisms and especially marine sponges.

Despite the significant variability observed in the present study for the "cleanup" metrics of sponges among different pollutants and species, the ranges of values obtained for clearance rates were similar to those that previously reported for the uptake of microalgae by the same sponge species (clearance rate: 0.4-7.0 mL h^{-1} g^{-1}; Varamogianni-Mamatsi et al., 2021). This means that the specific sponges can serve as efficient biofilters not only for the removal of POM and biological particles, but also for the mitigation of dissolved organic

351 pollutants that may enter aquatic environments as a result of intensive aquaculture practises.

352 **3.2. Removal of DOM by marine sponges**

353 Apart from individual organic pollutants, we further investigated the "cleanup" capacity of sponges against
354 complex mixtures of dissolved organic compounds. More specifically, two types of DOM, resulting from the
355 0.45μm-filtrate of sea bream excreta and a typical fish feed, were selected in an effort to simulate the DOM
356 present in the seawaters of fish aquaculture farms. **Fig. 2A-B** shows the concentration-time profiles of those
357 two DOM substrates over an 8-h exposure to the four sponge species. Although not as rapid as with individual
358 pollutants, a gradual decrease of both substrates was also evident for the majority of species, while the loss
359 of DOM in the control experiments was minimal. After 8-h of exposure, sponges retained 4-15% and 4-30%
360 of the DOM originating from fish feed and fish excreta, respectively. Once again, *A. oroides* stood out as the
361 best DOM uptaker among the four sponge species.

362 The decrease in DOM content was fairly described by the exponential model given in **Eq. (3)** (R^2: 0.79-0.95
363 and 0.88-0.97 for DOM of fish excreta and fish feed, respectively). However, a deviation was evident when
364 *A. oroides* was exposed to DOM from fish excreta. The latter showed a considerable decline over the first 5
365 h and then tended to an asymptotic value. Similar saturation patterns have been observed in a previous in
366 vitro study of Camacho et al. (2006), where explants of the poecilosclerid sponge *C. crambe* (Schmidt) were
367 exposed to various fructose concentrations.

368 The clearance and retention rates were calculated for the different sponge species and DOM substrates using
369 the experimental results from three explants. The average values are presented in **Table 3**. Significant
370 differences in the assimilation rates of the two substrates were tracked for *A. oroides* and *C. reniformis* (*t*-
371 test, $p<0.05$), with DOM from fish excreta providing higher values in both cases. In general, the clearance
372 rates derived for organic mixtures were in the lower range of those measured for individual organic pollutants
373 and approximated the values of pollutants of low hydrophobicity (i.e., OTC, diuron). Based on this finding, it
374 was hypothesized that DOM substrates were mainly composed of hydrophilic components that can be barely
375 assimilated by sponges. To test this possibility, the lipophilicity of both substrates was assessed using
376 reversed-phase HPLC, which separates the different molecules on the basis of their hydrophobic interactions
377 with the stationary phase. Indeed, the chromatograms of both DOM types comprised intense unresolved

peaks mainly eluting at retention times lower than 1.0 min (**Fig. S4** and **S5**). Considering that OTC and diuron eluted at 2.0 and 4.0 min, respectively, it can reasonably be inferred that DOM substrates were of hydrophilic nature, thus explaining their limited uptake from sponges.

To further investigate the cleanup behavior of sponges against complex organic mixtures and whether faster uptake can be achieved for DOM of greater lipophilic character, additional experiments were performed using a dilute solution of olive oil. The HPLC analysis of this substrate revealed major peaks eluting at higher retention times (2.25-3.71 min) than those observed in the chromatograms of fish feed and faeces (**Fig. S6**). Due to resource constraints, the specific "cleanup" tests were limited to the *A. oroides* explant presenting the highest removal rates for individual organic pollutants. **Fig. S7** displays the concentration-time profile of olive oil in experimental systems with or without *A. oroides*. Under the filtering action of sponge, the organic substrate showed a sharp decline and it was totally consumed within 5 hours. The corresponding clearance rate was estimated as 5.6 mL $h^{-1} g^{-1}$, outpacing the values measured for DOM of fish feed (0.2 mL $h^{-1} g^{-1}$) and fish excreta (0.5 mL $h^{-1} g^{-1}$), while it was close to the rate observed for the highly lipophilic phenanthrene (7.8 mL h^{-1} g^{-1}). These results corroborate that sponges are capable of uptaking multi-component organic mixtures from seawater and the speed of this process is determined by the overall lipophilic character of the mixture rather than its chemical complexity.

A comparison of our results with previous in vitro studies, dealing with complex mixtures uptake, is hard to be carried out. The primary reason concerns their limited number and their inconsistency with our target substrates. In the in vitro study of Fu et al. (2007), clones of the sponge *H. perlevis* were exposed to different concentrations of an algal/fish protein powder mixture for 24 h. However, this so-called dead organic matter diet was used as a representative of TOC pool in aquaculture settings, which means that besides DOC, the effect of particulate organic material was also taken under consideration. Such differences in the substrate nature of the latter study and ours, make the comparison of the results quite difficult. Leaving retention metrics aside, we can report that the clearance rates obtained from our study (0.1-0.5 mL $h^{-1} g^{-1}$ wet weight) fluctuated within the lower range of those measured for *H. perlevis* (0.33-7.64 mL $h^{-1} g^{-1}$ wet weight).

Additionally, the uptake of natural seawater DOM has been investigated in a handful of in situ studies, but few of them actually provide numeric insights into the respective sponge rates. By placing sponges in

405 incubation chambers and performing experiments at 12 m depth, de Goeij et al. (2008) investigated DOC
406 removal from three encrusting species. The clearance rates obtained from those in situ measurements were
407 126-702 mL h^{-1} g^{-1} for *Halisarca caerulea*, 180-330 for *Mycale microsigmatosa* and 168-210 for *Merlia*
408 *normani* (assuming sponge density of 1 g mL^{-1}), which are three orders of magnitude higher than ours.
409 Yahel et al. (2003) measured in situ the DOC content of the water inhaled and exhaled by the Indo-West
410 Pacific reef sponge *T. swinhoei* in an effort to unravel the "mystery" between metazoans and DOC cycling.
411 Their findings indicated an average carbon uptake of 26.0 nmol C mL_{sponge}^{-1} min^{-1}, which is equivalent to 18.7
412 μg C h^{-1} g^{-1}, assuming 1 g mL^{-1} sponge density. Despite the higher values, this is only twice the size from our
413 reported range for *A. oroides* and *C. reniformis* (9.0 μg C h^{-1} g^{-1}).
414 By following a similar, yet more improved sampling technique, Ribes et al. (2023) intended to investigate the
415 seasonal DOM uptake by four different sponges, including our case study species *A. oroides* and *C. reniformis*.
416 The average retention rates with respect to ambient DOC were estimated in the range of 17.9-107.3 μg h^{-1}
417 g^{-1} (assuming 1 g mL^{-1} sponge density). Considering that sponges are likely to function optimally when
418 present in their natural habitats, it is expected to receive lower cleaning metrics in a laboratory scale.

419 **3.3. Mechanistic insights into the uptake of dissolved organic pollutants by sponges**

420 Although many studies have brought into light the mechanisms by which sponges uptake POM (i.e, bacteria,
421 microalgae, latex spheres) (Reiswig, 1971; Turon et al., 1997), little is known about DOM assimilation. Some
422 microscopic observations suggested that sponge choanocytes are capable of capturing particles of very small
423 size, such as 0.1 μm latex beads (Leys and Eerkes-Medrano, 2006), which are operationally classified as
424 "dissolved" (i.e., material passing through 0.45-μm filters). The sieving function of choanocyte collars against
425 microparticles was underscored in that study, but actual information about the interactions of water-soluble
426 molecules with sponge surface was not provided. Other reports suggested the implication of microbial
427 symbionts in the uptake and transformation of DOM by sponges (particularly in species with high microbial
428 abundance; Olinger et al., 2021), but a symbiont-free uptake of DOC has been also supported (Gantt et al.,
429 2019).
430 Regardless of microbes contribution, it is obvious that the capture of dissolved organic molecules is dictated
431 by processes taking place in the aquiferous system of sponges. To the best of our knowledge, it is only

Norman et al. (2014) who thoroughly investigated the adsorption kinetics of carmine dye on sponges, but this study aimed at staining dry demosponge skeleton (sponginin) for biomedical applications rather than elucidating the uptake of dissolved compounds in the biosurface of living sponges. Nevertheless, carmine was shown to be readily adsorbed on proteinaceous sponge skeleton, but a small percentage of it (5 to 19%) could be desorbed back to fresh water.

Considering this fact, we conducted additional experiments to ascertain if organic pollutants uptake is governed by the active pumping/filtering of sponges or by simple passive adsorption on sponge biosurfaces and whether assimilated pollutants can return back to water due to desorption/excretion mechanisms. This investigation was limited to phenanthrene, which was one of the most readily assimilated compounds by the different sponge species.

3.3.1. Assessment of passive adsorption of pollutants onto sponge biosurfaces

To evaluate the contribution of passive adsorption of organic pollutants by sponge biosurfaces, one explant from each sponge species was sacrificed and the dead specimens were exposed to a phenanthrene solution. **Fig. 3** shows the concentration-time profile of phenanthrene in sponge and control experiments over the 8-h exposure period. In the presence of dead sponge explants, phenanthrene concentration showed a substantial decrease, which, after control subtraction, ranged between 16% (*A. cannabina*) and 37% (*A. oroides*) at the end of the experiments. However, these values are quite low when compared with the rapid removal of the same compound by living sponges (**Fig. 1E**). For example, the decrease of phenanthrene by living *A. oroides* reached 93% and 99% after just 1 and 3 hours of exposure, respectively.

Similarly to the procedure described above, the data from sponge experiments were fitted to a first-order kinetic model (**Eq. (3)**) and the influence of controls was subtracted to derive equations simulating the net effect of phenanthrene adsorption by sponges (**Fig. S8**). In all cases, the model provided good fit to experimental data (R^2: 0.79-0.96). In analogy to retention rates, the adsorption rates of phenanthrene were derived using the modeled data. A comparison of retention rates resulting from passive adsorption effects and active pumping/filtration activity of sponges, is presented in **Table 4**. Since the active uptake of phenanthrene by *A. oroides* was almost completed within the first 1 h (**Fig. 1E**), all rates were calculated for the same time period to maintain consistency. The retention rates obtained for phenanthrene adsorption on

sponges were relatively low and indicated limited variability among the different species (33 to 58 ng of pollutant retained per hour and gram of sponge wet weight). For three of the study sponge species, these values were 6 to 10 times lower than the respective rates calculated above for active uptake (224 to 430 ng h^{-1} g^{-1}), while a 3-fold difference was observed for *S. foetidus* (active uptake: 112 ng h^{-1} g^{-1}).

Based on our findings, DOM uptake by sponges is mainly a biological process that can be reasonably attributed to the function of choanocytes. These flagellated cells line the microcavities in the canal system of sponges and represent their basic pumping and filtering units. The morphological and functional similarities of choanocytes with their closest protistan relatives, the choanoflagellates, have been discussed for centuries (Mah et al., 2014). The latter have long been documented to ingest a variety of dissolved organic compounds, including carbohydrates, proteins, components of the colloidal fraction of DOM (Tranvik et al., 1993), as well as high molecular weight polysaccharide dextrans (Marchant and Scott, 1993; Sherr, 1988). Given this fact, it is not surprising that sponges are capable of removing DOM and individual chemicals from seawater. In both cell types, the capturing mechanism should involve flagellum-mediated diffusion of the organic solutes from the bulk liquid to the base of choanocyte collars and their subsequent pinocytosis through the formation of intracellular vacuoles (Hickman et al., 2003). Sponge microbial symbionts have been also suggested to play a role in the retention of heavy metals by sponges (Gravina et al., 2022), but their contribution to the uptake of dissolved organic pollutants remains unclear. Additional studies are required to reveal whether diffusion, pinocytosis, sponge-associated microbiota activity, or a combination of all of them, control the overall kinetics of DOM uptake by sponges.

3.3.2. Assessment of pollutants desorption/release from sponges to seawater

Although active pumping/filtration was found to dictate dissolved pollutants uptake by sponges, it is not clear if this process is reversible and whether entrapped pollutants can return back to water through desorption or excretory phenomena. To investigate this aspect, explants of the four study sponges were exposed to phenanthrene solution for sufficient time (i.e., 16 h) to enable pollutant uptake and subsequently immersed in pollutant-free NSW. By monitoring pollutant levels for the following 8 h, release of phenanthrene was assessed. **Fig. 4** illustrates the percentage of sponge-uptaken phenanthrene that was returned back to clean NSW over the course of experiments. For all sponges, the levels of desorbing/excreting phenanthrene

demonstrated a gradual increase during the first 3 h, followed by quite steady values. The species *A. cannabina* and *C. reniformis* showed a more pronounce release, which approached 3% by the end of the experiments, while slightly lower values were observed for *A. oroides* and *S. foetidus* (~1.5%). Despite minor discrepancies, our findings suggest that organic pollutants uptaken by marine sponges are strongly attached on their aquiferous system and they can be hardly released back to the surrounding environment.

4. Conclusion

The present study focused on the uptake of dissolved organic substances by marine sponges. The kinetics of this process were investigated for four widespread Mediterranean sponges against a series of aquaculture-related organic pollutants and complex mixtures, in an effort to explore the potential of these biofiltering organisms for the alleviation of pollution near fish farms. All species were able to assimilate individual pollutants belonging to antibiotics, antifouling biocides and PAHs, but the speed varied significantly among compounds. With regard to multi-component organic mixtures, sponges retained DOM from fish feed and fish excreta at moderate rates, while a much faster DOM uptake was observed for a solution of olive oil composed of more lipophilic compounds. The species *A. oroides* exhibited the greatest filtering performance over all individual pollutants and DOM substrates, with the highest rates being recorded for the polyaromatic hydrocarbons 2,6-DMN and phenanthrene. In all four sponges, a pronounced preference for highly lipophilic compounds was discovered and all “cleanup” metrics demonstrated a strong positive correlation with the hydrophobicity of the compounds. To a further extent, we showed that active water pumping/filtration of sponges is the major driving force behind the removal of dissolved pollutants from seawater, as the uptake rates resulting from this mechanism were much higher than those derived for passive adsorption of pollutants onto the surface of dead sponges. Last, but not least, our results showed that the organic pollutants uptaken by sponges can barely be released back to seawater via desorption or excretory mechanisms, implying that the chemicals were strongly retained into the inner sponge body.

Sponges have been proved so far to be one of the most promising candidates for bioremediation in integrated multitrophic aquaculture systems. Apart from the well-known ability of sponges in removing POM, our study provides extensive evidence that these invertebrates are also very effective DOC uptakers and hold great

potential as bioremediators in fish farms or other areas impacted by excessive organic/chemical loadings. Further field investigations and in situ trials are warranted to verify the fast uptake kinetics of sponges for dissolved organic pollutants under real-life conditions.

Declaration of Competing Interest

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

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References

Aresta, A., Nonnis Marzano, C., Lopane, C., Corriero, G., Longo, C., Zambonin, C., Stabili, L., 2015. Analytical investigations on the lindane bioremediation capability of the demosponge *Hymeniacidon perlevis*. Marine Pollution Bulletin 90, 143–149. https://doi.org/10.1016/j.marpolbul.2014.11.003.

Buma, A.G.J., Sjollema, S.B., van de Poll, W.H., Klamer, H.J.C., Bakker, J.F., 2009. Impact of the antifouling agent Irgarol 1051 on marine phytoplankton species. Journal of Sea Research 61, 133–139. https://doi.org/10.1016/j.seares.2008.11.007.

Camacho, F.G., Chileh, T., García, M.C.C., Mirón, A.S., Belarbi, E.H., Gómez, A.C., Grima, E.M., 2006. Sustained growth of explants from Mediterranean sponge *Crambe crambe* cultured in vitro with enriched RPMI 1640. Biotechnology Progress 22, 781–790. https://doi.org/10.1021/bp050341m.

Claus, G., Madri, P., Kunen, S., 1967. Removal of microbial pollutants from waste effluents by the redbeard sponge. Nature 216, 712–714. https://doi.org/10.1038/216712a0.

Coughlan, J., 1969. The estimation of filtering rate from the clearance of suspensions. Marine Biology 2, 356–358. https://doi.org/10.1007/BF00355716.

de Goeij, J.M., van den Berg, H., van Oostveen, M.M., Epping, E.H.G., van Duyl, F.C., 2008. Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. Marine Ecology Progress Series 357, 139–151. https://doi.org/10.3354/meps07403.

de Goeij, J.M., van Oevelen, D., Vermeij, M.J.A., Osinga, R., Middelburg, J.J., de Goeij, A.F.P.M., Admiraal, W., 2013. Surviving in a marine desert: The sponge loop retains resources within coral reefs. Science 342, 108–110. https://doi.org/10.1126/science.1241981.

Ding, Y., Wu, Z., Zhang, R., Yu, K., Wang, Y., Zou, Q., Zeng, W., Han, M., 2019. Organochlorines in fish from the coastal coral reefs of Weizhou Island, south China sea: Levels, sources, and bioaccumulation. Chemosphere 232, 1–8. https://doi.org/10.1016/j.chemosphere.2019.05.199.

ECHA, 2018. CLH report for 1,4-dimethylnaphthalene in proposal for harmonised classification and labelling based on regulation (EC) No 1272/2008, Annex VI, Part 2, European Chemicals Agency, Helsinki.

Endicott, D., Kreis, R.G., Mackelburg, L., Kandt, D., 1998. Modeling PCB bioaccumulation by the zebra mussel (*Dreissena polymorpha*) in Saginaw Bay, Lake Huron. Journal of Great Lakes Research 24, 411–426. https://doi.org/10.1016/S0380-1330(98)70832-9.

FAO, 2020. The state of world fisheries and aquaculture 2020, Sustainability in action, Rome. https://doi.org/10.4060/ca9229en.

Ferguson, J.C., 1980. Fluxes of dissolved amino acids between sea water and *Echinaster*. Comparative Biochemistry and Physiology Part A: Physiology 65, 291–295. https://doi.org/10.1016/0300-9629(80)90031-6.

Ferretti, C., Vacca, S., Ciucis, C.D., Marengo, B., Duckworth, A.R., Manconi, R., Pronzato, R., Domenicotti, C., 2009. Growth dynamics and bioactivity variation of the Mediterranean demosponges *Agelas oroides* (Agelasida, Agelasidae) and *Petrosia ficiformis* (Haplosclerida, Petrosiidae). Marine Ecology 30, 327–336. https://doi.org/10.1111/j.1439-0485.2008.00278.x.

Frost, T.M., 1978. In situ measurements of clearance rates for the freshwater sponge *Spongilla lacustris*. Limnology and Oceanography 23, 1034–1039. https://doi.org/10.4319/lo.1978.23.5.1034.

Fu, W., Sun, L., Zhang, X., Zhang, W., 2006. Potential of the marine sponge *Hymeniacidon perleve* as a bioremediator of pathogenic bacteria in integrated aquaculture ecosystems. Biotechnology and Bioengineering 93, 1112–1122. https://doi.org/10.1002/bit.20823.

Fu, W., Wu, Y., Sun, L., Zhang, W., 2007. Efficient bioremediation of total organic carbon (TOC) in integrated aquaculture system by marine sponge *Hymeniacidon perleve*. Biotechnology and Bioengineering 97, 1387–1397. https://doi.org/10.1002/bit.21352.

Gantt, S.E., McMurray, S.E., Stubler, A.D., Finelli, C.M., Pawlik, J.R., Erwin, P.M., 2019. Testing the relationship between microbiome composition and flux of carbon and nutrients in Caribbean coral reef sponges. Microbiome 7, Article 124. https://doi.org/10.1186/s40168-019-0739-x.

Gentric, C., Rehel, K., Dufour, A., Sauleau, P., 2016. Bioaccumulation of metallic trace elements and organic pollutants in marine sponges from the South Brittany Coast, France. Journal of Environmental Science and Health, Part A 51, 213–219. https://doi.org/10.1080/10934529.2015.1094327.

Gerovasileiou, V., Chintiroglou, C., Vafidis, D., Koutsoubas, D., Sini, M., Dailianis, T., Issaris, Y., Akritopoulou, E., Dimarchopoulou, D., Voultsiadou, E., 2015. Census of biodiversity in marine caves of the eastern Mediterranean Sea. Mediterranean Marine Science 16, 245–265. https://doi.org/10.12681/mms.1069.

Geyer, H.J., Scheunert, I., Brüggemann, R., Steinberg, C., Korte, F., Kettrup, A., 1991. QSAR for organic chemical bioconcentration in Daphnia, algae, and mussels. Science of The Total Environment, QSAR in Environmental Toxicology 109–110, 387–394. https://doi.org/10.1016/0048-9697(91)90193-I.

Gökalp, M., Mes, D., Nederlof, M., Zhao, H., Merijn de Goeij, J., Osinga, R., 2021. The potential roles of sponges in integrated mariculture. Reviews in Aquaculture 13, 1159–1171. https://doi.org/10.1111/raq.12516.

Gravina, M.F., Longo, C., Puthod, P., Rosati, M., Colozza, N., Scarselli, M., 2022. Heavy metal accumulation capacity of *Axinella damicornis* (Esper, 1794) (Porifera, Demospongiae): A tool for bioremediation of polluted seawaters. Mediterranean Marine Science 23, 125-133. https://doi.org/10.12681/mms.27792.

Hadas, E., Marie, D., Shpigel, M., Ilan, M., 2006. Virus predation by sponges is a new nutrient-flow pathway in coral reef food webs. Limnology and Oceanography 51, 1548–1550. https://doi.org/10.4319/lo.2006.51.3.1548.

Hickman, C. P., Roberts, L. S., Larson, A., 2003. Sponges: Phylum Porifera, Animal Diversity, 3rd Edition. New York, McGraw-Hill.

Idan, T., Shefer, S., Feldstein, T., Yahel, R., Huchon, D., Ilan, M., 2018. Shedding light on an East-Mediterranean mesophotic sponge ground community and the regional sponge fauna. Mediterranean Marine Science 19, 84–106. https://doi.org/10.12681/mms.13853.

Idan, T., Goren, L., Shefer, S., Ilan, M., 2020. Sponges in a changing climate: Survival of *Agelas oroides* in a warming Mediterranean Sea. Frontiers in Marine Science 7, Article 603593. https://doi.org/10.3389/fmars.2020.603593.

Koukouras, A., Russo, A., Voultsiadou-Koukoura, E., Arvanitidis, C., Stefanidou, D., 1996. Macrofauna associated with sponge species of different morphology. Marine Ecology 17, 569–582. https://doi.org/10.1111/j.1439-0485.1996.tb00418.x.

Laport, M.S., Pontes, P.V.M., dos Santos, D.S., Santos-Gandelman, J. de F., Muricy, G., Bauwens, M., Giambiagi-deMarval, M., George, I., 2016. Antibiotic resistance genes detected in the marine sponge *Petromica citrina* from Brazilian coast. Brazilian Journal of Microbiology 47, 617–620. https://doi.org/10.1016/j.bjm.2016.04.016.

Leys, S.P., Eerkes-Medrano, D.I., 2006. Feeding in a calcareous sponge: Particle uptake by pseudopodia. Biological Bulletin 211, 157–171. https://doi.org/10.2307/4134590.

Longo, C., Corriero, G., Licciano, M., Stabili, L., 2010. Bacterial accumulation by the Demospongiae *Hymeniacidon perlevis*: a tool for the bioremediation of polluted seawater. Marine Pollution Bulletin 60, 1182–1187. https://doi.org/10.1016/j.marpolbul.2010.03.035.

Longo, C., Pierri, C., Mercurio, M., Trani, R., Cardone, F., Carbonara, P., Alfonso, S., Stabili, L., 2022. Bioremediation capabilities of *Hymeniacidon perlevis* (Porifera, Demospongiae) in a land-based experimental fish farm. Journal of Marine Science and Engineering 10, 874. https://doi.org/10.3390/jmse10070874.

Mah, J.L., Christensen-Dalsgaard, K.K., Leys, S.P., 2014. Choanoflagellate and choanocyte collar-flagellar systems and the assumption of homology. Evolution & Development 16, 25–37. https://doi.org/10.1111/ede.12060.

Maldonado, M., Zhang, X., Cao, X., Xue, L., Cao, H., Zhang, W., 2010. Selective feeding by sponges on

621 pathogenic microbes: A reassessment of potential for abatement of microbial pollution. Marine Ecology
622 Progress Series 403, 75–89. https://doi.org/10.3354/meps08411.
623 Manconi, R., Cadeddu, B., Ledda, F., Pronzato, R., 2013. An overview of the Mediterranean cave-dwelling
624 horny sponges (Porifera, Demospongiae). ZooKeys 281, 1–68. https://doi.org/10.3897/zookeys.281.4171.
625 Marchant, H.J., Scott, F.J., 1993. Uptake of sub-micrometre particles and dissolved organic material by
626 Antarctic choanoflagellates. Marine Ecology Progress Series 92, 59–64.
627 https://www.jstor.org/stable/24832616.
628 Milanese, M., Chelossi, E., Manconi, R., Sarà, A., Sidri, M., Pronzato, R., 2003. The marine sponge *Chondrilla*
629 *nucula* Schmidt, 1862 as an elective candidate for bioremediation in integrated aquaculture. Biomolecular
630 Engineering 20, 363–368. https://doi.org/10.1016/S1389-0344(03)00052-2.
631 Nasher, E., Heng, L.Y., Zakaria, Z., Surif, S., 2013. Concentrations and sources of polycyclic aromatic
632 hydrocarbons in the seawater around Langkawi Island, Malaysia. Journal of Chemistry 2013, Article 975781.
633 https://doi.org/10.1155/2013/975781.
634 Nickel, M., Brümmer, F., 2003. In vitro sponge fragment culture of *Chondrosia reniformis* (Nardo, 1847).
635 Journal of Biotechnology 100, 147–159. https://doi.org/10.1016/s0168-1656(02)00256-0.
636 Norman, M., Bartczak, P., Zdarta, J., Tylus, W., Szatkowski, T., Stelling, A.L., Ehrlich, H., Jesionowski, T., 2014.
637 Adsorption of C.I. Natural Red 4 onto spongin skeleton of marine demosponge. Materials 8, 96–116.
638 https://doi.org/10.3390/ma8010096.
639 Ogura, N., 1972. Rate and extent of decomposition of dissolved organic matter in surface seawater. Marine
640 Biology 13, 89–93. https://doi.org/10.1007/BF00366559.
641 Olinger, L.K., Strangman, W.K., McMurray, S.E., Pawlik, J.R., 2021. Sponges with microbial symbionts
642 transform dissolved organic matter and take up organohalides. Frontiers in Marine Science 8, Article 665789.
643 https://doi.org/10.3389/fmars.2021.665789.
644 Oliver, B.G., Niimi, A.J., 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other
645 chlorinated hydrocarbons in the Lake Ontario ecosystem. Environmental Science & Technology 22, 388–397.
646 https://doi.org/10.1021/es00169a005.
647 Osinga, R., Kleijn, R., Groenendijk, E., Niesink, P., Tramper, J., Wijffels, R.H., 2001. Development of in vivo

sponge cultures: Particle feeding by the tropical sponge *Pseudosuberites* aff. *andrewsi*. Marine Biotechnology 3, 544–554. https://doi.org/10.1007/s1012601-0078-2.

Perez, T., Wafo, E., Fourt, M., Vacelet, J., 2003. Marine sponges as biomonitor of polychlorobiphenyl contamination: Concentration and fate of 24 congeners. Environmental Science & Technology 37, 2152–2158. https://doi.org/10.1021/es026234v.

Phelan, R.W., Clarke, C., Morrissey, J.P., Dobson, A.D.W., O'Gara, F., Barbosa, T.M., 2011. Tetracycline resistance-encoding plasmid from *Bacillus* sp. strain #24, isolated from the marine sponge *Haliclona simulans*. Applied and Environmental Microbiology 77, 327–329. https://doi.org/10.1128/AEM.01239-10.

Pronzato, R., Cerrano, C., Cubeddu, T., Lanza, S., Magnino, G., Manconi, R., Pantelis, J., Sara, A., Sidri, M., 1998. Sustainable development in coastal areas: role of sponge farming in integrated aquaculture. Aquaculture and Water: Fish Culture, Shellfish Culture and Water Usage, 231–232. https://doi.org/10.13140/2.1.2461.3768.

Pronzato, R., Bavestrello, G., Cerrano, C., Magnino, G., Manconi, R., Pantelis, J., Sarà, A., Sidri, M., 1999. Sponge farming in the Mediterranean Sea: new perspectives. Memoirs of the Queensland Museum 44, 485–491.

Reiswig, H.M., 1971. Particle feeding in natural populations of three marine demosponges. Biological Bulletin 141, 568–591. https://doi.org/10.2307/1540270.

Ribes, M., Coma, R., Gili, J.-M., 1999. Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. Marine Ecology Progress Series 176, 179–190. https://doi.org/10.3354/meps176179.

Ribes, M., Yahel, G., Romera-Castillo, C., Mallenco, R., Morganti, T.M., Coma, R., 2023. The removal of dissolved organic matter by marine sponges is a function of its composition and concentration: An in situ seasonal study of four Mediterranean species. Science of The Total Environment 871, Article 161991. https://doi.org/10.1016/j.scitotenv.2023.161991.

Riisgård, H.U., Thomassen, S., Jakobsen, H., Larsen, P., 1993. Suspension-feeding in marine sponges *Halichondria Panicea* and *Haliclona Urceolus*: Effects of temperature on filtration rate and energy cost of pumping. Marine Ecology Progress Series 96, 177–188. https://doi.org/10.3354/meps096177.

675 Rosa, J., Lemos, M.F.L., Crespo, D., Nunes, M., Freitas, A., Ramos, F., Pardal, M.Â., Leston, S., 2020. Integrated
676 multitrophic aquaculture systems – Potential risks for food safety. Trends in Food Science & Technology 96,
677 79–90. https://doi.org/10.1016/j.tifs.2019.12.008.
678 Sakkas, V.A., Lambropoulou, D.A., Albanis, T.A., 2002. Photochemical degradation study of Irgarol 1051 in
679 natural waters: influence of humic and fulvic substances on the reaction. Journal of Photochemistry and
680 Photobiology A: Chemistry 147, 135–141. https://doi.org/10.1016/S1010-6030(01)00623-2.
681 Sherr, E.B., 1988. Direct use of high molecular weight polysaccharide by heterotrophic flagellates. Nature
682 335, 348–351. https://doi.org/10.1038/335348a0.
683 Smaldone, G., Marrone, R., Cappiello, S., Martin, G.A., Oliva, G., Cortesi, M.L., Anastasio, A., 2014. Occurrence
684 of antibiotic resistance in bacteria isolated from seawater organisms caught in Campania Region: preliminary
685 study. BMC Veterinary Research 10, Article 161. https://doi.org/10.1186/1746-6148-10-161.
686 Stabili, L., Licciano, M., Giangrande, A., Longo, C., Mercurio, M., Marzano, C.N., Corriero, G., 2006. Filtering
687 activity of *Spongia officinalis* var. *adriatica* (Schmidt) (Porifera, Demospongiae) on bacterioplankton:
688 Implications for bioremediation of polluted seawater. Water Research 40, 3083–3090.
689 https://doi.org/10.1016/j.watres.2006.06.012.
690 Stabili, L., Licciano, M., Longo, C., Corriero, G., Mercurio, M., 2008. Evaluation of microbiological
691 accumulation capability of the commercial sponge *Spongia officinalis* var. *adriatica* (Schmidt) (Porifera,
692 Demospongiae). Water Research 42, 2499–2506. https://doi.org/10.1016/j.watres.2008.02.008.
693 Stephens, G.C., Schinske, R.A., 1961. Uptake of amino acids by marine invertebrates. Limnology and
694 Oceanography 6, 175–181. https://doi.org/10.4319/lo.1961.6.2.0175.
695 Tornero, V., Hanke, G., 2016. Chemical contaminants entering the marine environment from sea-based
696 sources: A review with a focus on European seas. Marine Pollution Bulletin 112, 17–38.
697 https://doi.org/10.1016/j.marpolbul.2016.06.091.
698 Tranvik, L.J., Sherr, E.B., Sherr, B.F., 1993. Uptake and utilization of “colloidal DOM” by heterotrophic
699 flagellates in seawater. Marine Ecology Progress Series 92, 301–309. https://doi.org/10.3354/meps092301.
700 Turon, X., Galera, J., Uriz, M.J., 1997. Clearance rates and aquiferous systems in two sponges with contrasting
701 life-history strategies. Journal of Experimental Zoology 278, 22–36. https://doi.org/10.1002/(SICI)1097-

010X(19970501)278:1<22::AID-JEZ3>3.0.CO;2-8.

Van Well, P.B., 1949. On the physiology of the tropical fresh water sponge, *Spongilla proliferens* Annand; ingestion, digestion and excretion. Physiologia comparata et oecologia 1, 110-126.

Varamogianni-Mamatsi, D., Anastasiou, T.I., Vernadou, E., Papandroulakis, N., Kalogerakis, N., Dailianis, T., Mandalakis, M., 2021. A multi-species investigation of sponges' filtering activity towards marine microalgae. Marine Drugs 20, Article 24. https://doi.org/10.3390/md20010024.

Voultsiadou, E., 2005. Demosponge distribution in the eastern Mediterranean: a NW–SE gradient. Helgoland Marine Research 59, 237–251. https://doi.org/10.1007/s10152-005-0224-8.

Wehrl, M., Steinert, M., Hentschel, U., 2007. Bacterial uptake by the marine sponge *Aplysina aerophoba*. Microbial Ecology 53, 355–365. https://doi.org/10.1007/s00248-006-9090-4.

Weissenfels, N., 1992. The filtration apparatus for food collection in freshwater sponges (Porifera, Spongillidae). Zoomorphology 112, 51–55. https://doi.org/10.1007/BF01632994.

Wilkinson, C.R., Vacelet, J., 1979. Transplantation of marine sponges to different conditions of light and current. Journal of Experimental Marine Biology and Ecology 37, 91–104. https://doi.org/10.1016/0022-0981(79)90028-5.

XiaoWu, Shao, Y., 2017. Study of kinetics mechanism of PAHs photodegradation in solution. Procedia Earth and Planetary Science, 15th Water-Rock Interaction International Symposium, WRI-15 17, 348–351. https://doi.org/10.1016/j.proeps.2016.12.088.

Yahel, G., Sharp, J.H., Marie, D., Häse, C., Genin, A., 2003. In situ feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon. Limnology and Oceanography 48, 141–149. https://doi.org/10.4319/lo.2003.48.1.0141.

Yebra, D., Kiil, S., Dam-Johansen, K., 2004. Antifouling technology - Past, present and future steps towards efficient and environmentally friendly antifouling coatings. Progress in Organic Coatings 50, 75–104. https://doi.org/10.1016/j.porgcoat.2003.06.001.

Zhang, X., Zhang, W., Xue, L., Zhang, B., Jin, M., Fu, W., 2010. Bioremediation of bacteria pollution using the marine sponge *Hymeniacidon perlevis* in the intensive mariculture water system of turbot *Scophthalmus maximus*. Biotechnology and Bioengineering 105, 59–68. https://doi.org/10.1002/bit.22522.

Table 1. The studied organic substrates and their role in aquaculture settings along with their octanol-water partition coefficients ($LogK_{OW}$).

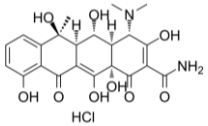
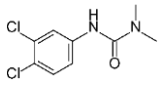
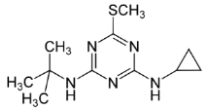
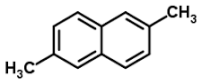
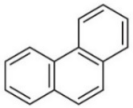
Chemical pollutant (Trivial name)	Chemical structure	Use/Origin	$logK_{ow}$
Oxytetracycline		Antibiotic with worldwide use in fish farming	-1.12 (Daghrir and Drogui, 2013)
3-(3,4-dichlorophenyl)1,1-dimethylurea (Diuron)		Non-metallic organic booster biocide widely used in marine antifouling paints	2.82 (Konstantinou and Albanis, 2004)
2-methylthio-4-butylamino-6-cyclopropylamine-s-triazine (Irgarol 1051)		Non-metallic organic booster biocide widely used in marine antifouling paints	3.95 (Loos, 2012)
2,6-Dimethylnaphthalene		Dicyclic aromatic hydrocarbon arising from combustion or oil anthropogenic activities	4.31 (Miller et al., 1985)
Phenanthrene		Tricyclic aromatic hydrocarbon arising from combustion or oil anthropogenic activities	4.57 (Miller et al., 1985)

Table 2. Clearance and retention rates of the four study sponge species derived for five organic pollutants with different levels of lipophilicity (i.e., $LogK_{OW}$).

	Clearance Rate, *c* (mL h^{-1} g_{sponge}^{-1})				Retention Rate, *r* ($ng_{chemical}$ h^{-1} g_{sponge}^{-1})			
Chemical	***A. oroides***	***A. cannabina***	***C. reniformis***	***S. foetidus***	***A. oroides***	***A. cannabina***	***C. reniformis***	***S. foetidus***
OTC	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	5 ± 2	8 ± 4	4 ± 0	15 ± 6
Diuron	0.8 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.0	125 ± 35	59 ± 12	30 ± 5	74 ± 27
Irgarol 1051	1.2 ± 0.1	0.7 ± 0.2	0.8 ± 0.2	2.0 ± 0.4	105 ± 9	60 ± 22	76 ± 15	165 ± 32
2,6-DMN	10.0 ± 1.3	4.5 ± 1.2	3.1 ± 1.2	2.2 ± 2.1	383 ± 69	288 ± 80	196 ± 75	148 ± 122
Phenanthrene	7.8 ± 1.7	4.3 ± 0.7	3.3 ± 1.1	1.4 ± 0.3	324 ± 74	283 ± 45	200 ± 67	96 ± 17

Table 3. Clearance and retention rates of the four study sponge species for two different types of dissolved organic matter obtained from fish feed and feces.

	Clearance Rate, *c* (mL h^{-1} g_{sponge}^{-1})				Retention Rate, *r* (μg_{DOC} h^{-1} g_{sponge}^{-1})			
DOM source	***A. oroides***	***A. cannabina***	***C. reniformis***	***S. foetidus***	***A. oroides***	***A. cannabina***	***C. reniformis***	***S. foetidus***
Fish feed	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	6 ± 1	7 ± 1	4 ± 0	7 ± 3
Fish feces	0.5 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	9 ± 0	4 ± 0	9 ± 0	3 ± 0

Table 4. Retention rates (in $ng_{chemical}\ h^{-1}\ g_{sponge}^{-1}$) attributed to passive adsorption and pumping activity of the four study sponge species against phenanthrene.

Retention process	*A. oroides*	*A. cannabina*	*C. reniformis*	*S. foetidus*
Passive adsorption	45	58	33	40
Active uptake	430	329	224	112

Figure Legends

Figure 1. Concentration-time profiles (mean values ± SD) of (A) OTC, (B) diuron, (C) Irgarol 1051, (D) 2,6-DMN and (E) phenanthrene in the treatment (i.e., under the presence of the species *A. oroides*, *A. cannabina*, *C. reniformis* and *S. foetidus*) and control experiments (i.e., without sponges) over the course of 8 h.

Figure 2. Concentration-time profiles (mean values ± SD) of two representative aquaculture DOM forms; (A) fish feed and (B) fish faeces, in the treatment (i.e., under the presence of the species *A. oroides*, *A. cannabina*, *C. reniformis* and *S. foetidus*) and control experiments (i.e., without sponges) over the course of 8 h.

Figure 3. Concentration-time profiles of phenanthrene in the treatment (i.e., under the presence of dead *A. oroides*, *A. cannabina*, *C. reniformis* and *S. foetidus* fragments) and control experiments (i.e., without sponges) over the course of 8 h, when assessing passive adsorption phenomena onto sponges biosurface.

Figure 4. Release percentage of uptaken phenanthrene by *A. oroides*, *A. cannabina*, *C. reniformis* and *S. foetidus* over the course of 8 h.

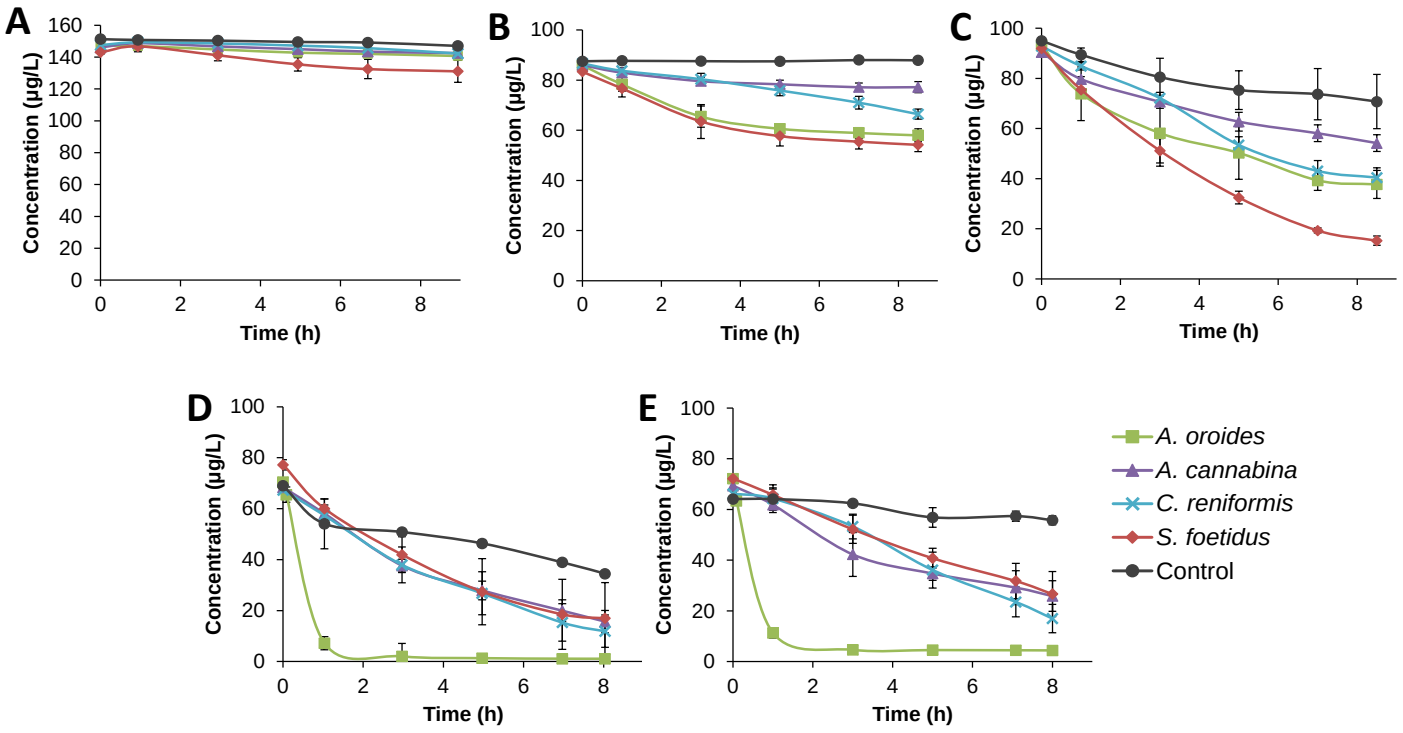

A
B
C
D
E
Concentration (µg/L)
Time (h)
A. oroides
A. cannabina
C. reniformis
S. foetidus
Control

Fig. 1.

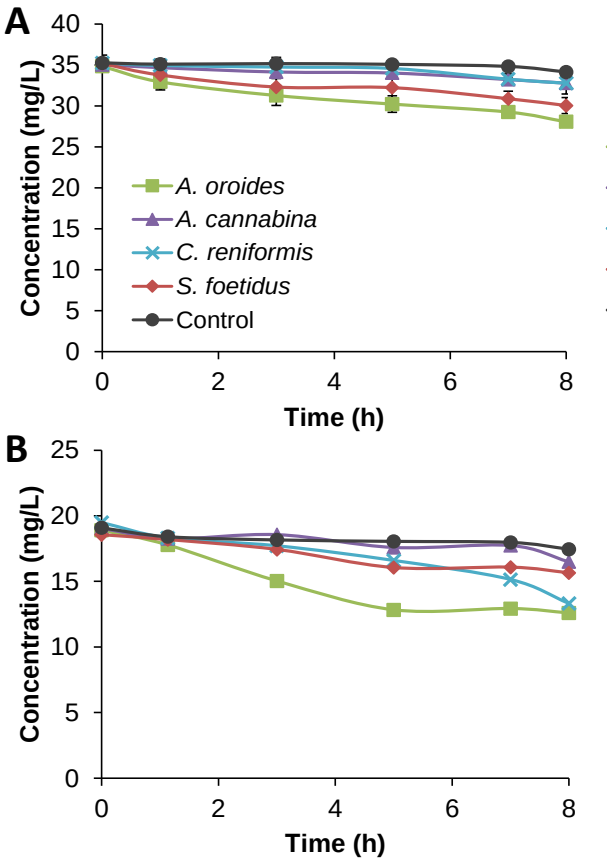

A
Concentration (mg/L)
40
35
30
25
20
15
10
5
0
0
2
4
6
8
Time (h)
A. oroides
A. cannabina
C. reniformis
S. foetidus
Control
B
Concentration (mg/L)
25
20
15
10
5
0
0
2
4
6
8
Time (h)

Fig. 2.

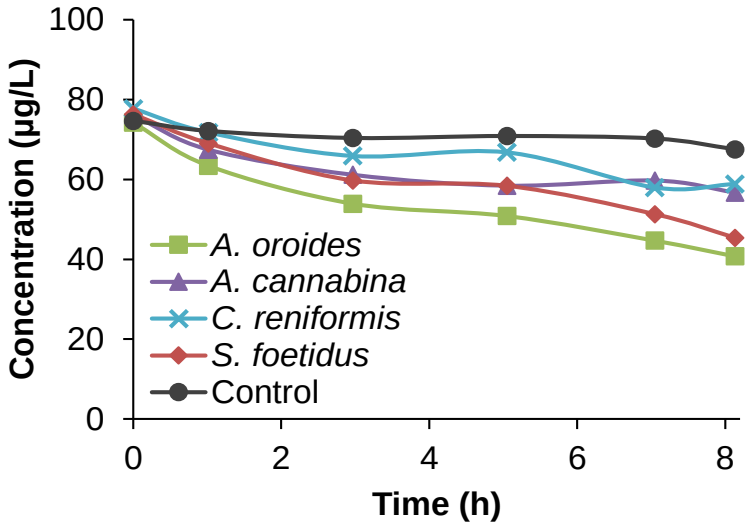

100
80
60
40
20
0
Concentration (µg/L)
0
2
4
6
8
Time (h)
A. oroides
A. cannabina
C. reniformis
S. foetidus
Control

Fig. 3.

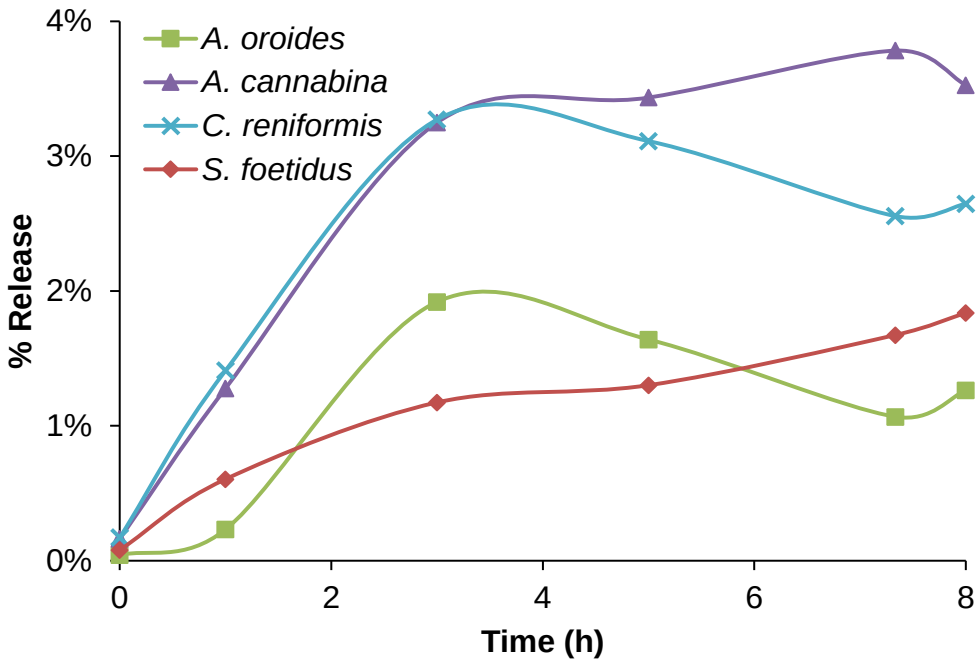

4%
3%
2%
1%
0%
% Release
A. oroides
A. cannabina
C. reniformis
S. foetidus
0
2
4
6
8
Time (h)

Fig. 4.

Highlights

- Marine sponges efficiently remove aquaculture-related dissolved organic pollutants
- *A. oroides* is the best-performing sponge species for pollutants uptake
- Sponge clearance rate is highly correlated with pollutants hydrophobicity
- Active pumping is the predominant mechanism for DOM assimilation by sponges
- Organic pollutants uptaken by sponges can hardly be released back to seawater