Biomonitoring of human activities recovery following lockdown in a highly touristic Mediterranean Island using *Mytilus galloprovincialis*

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ARTICLE INFO

ABSTRACT

Coastal waters face significant anthropogenic stress, particularly from tourism, exacerbating pollution, especially in areas like touristic islands. Ischia, the largest island in the Gulf of Naples and part of the Regno di Nettuno Marine Protected Area, suffers from pollution due to tourism and maritime traffic. During the initial SARS-CoV-2 lockdown from March to June 2020, Ischia was isolated, providing a unique opportunity to study pollutant release and its impact on coastal ecosystems. Adult *Mytilus galloprovincialis* mussels were transplanted to three sites on the island for active biomonitoring. Accumulation of chemicals in tissues and biomarkers related to metabolism, detoxification, and oxidative stress were measured. Results indicated that pollutants from daily activities entered the sea, affecting filter feeders. Translocated organisms showed modulated metabolic functions and biochemical changes, highlighting coastal vulnerability and calling for conservation efforts.

1. Introduction

In the last century coastal environments, due to the increasing population density and related activities, have undergone a very strong anthropization. In many areas, this process has considerably modified and altered the environment, threatening marine ecosystems and human health (Landrigan et al., 2020). Among anthropogenic disturbances, marine pollution threatens the marine life and the health of about three billion people worldwide (Landrigan et al., 2020). In particular, the Mediterranean coastal habitats are among the most endangered as a result of multiple and interacting processes (Halpern et al., 2008) representing 32 % of threatened habitats within EU countries. Nevertheless, people historically benefit directly or indirectly from these habitats due to food provision, and incomes from coastal tourism (Halpern et al., 2008). The degradation of coastal environments is thus causing great economic losses and preventing the achievement of the UN Sustainable Development Goals (2; 3; 6; 11; 14).

Along the Italian coast, the coastal area around the municipality of Naples including the surrounding islands makes a good example of a place where different interacting factors such as industrialization, fishery, over-exploitation, overpopulation, pollution and mass tourisms determine detrimental effects on the marine environment (Appolloni et al., 2018a, 2018b; Ferrigno et al., 2018; Pieretti et al., 2020; Barrenechea Angeles et al., 2023).

Several studies have reported high concentrations of heavy metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in marine sediments (Menghan et al., 2015; Trifuoggi et al., 2017; Morroni et al., 2020) and seafood (Naso et al., 2005; Fasano et al., 2018; Esposito et al., 2020; Morroni et al., 2020) from the Gulf of Naples, Pozzuoli and Salerno and in the area of Ischia Island (e.g., Warnau et al., 1995; Pergent-Martini, 1998; Pergent and Pergent-Martini, 1999; Guidetti and Fabiano, 2000; Tomicic et al., 2001). On the contrary there is a warring gap of knowledge about the presence of emerging contaminants such as pharmaceuticals, which encompass both prescribed and over-the counter medicines, and personal care products, which include disinfectants, fragrances, insect repellents, preservatives and UV filters, among others, albeit these substances can affect marine organisms' physiology, growth and reproduction, being designed to

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have a biological effect (Fent et al., 2006; Munari et al., 2014, 2016, 2018, 2019, 2020a, 2020b, 2022).

Nevertheless, the island (hosting around 60,000 inhabitants) is characterized by several forms of anthropogenic pressure, including mass tourism (more than a million tourists per year, mainly between June and September), and naval traffic. Under such a scenario, the absence of modern sewage treatment plans (Fig. S1), indicates that it is highly likely that there is a release of these chemicals directly into the marine environment. Thus, it is pivotal to check their occurrence and concentrations to evaluate the chemical load of the aquatic environment.

The advent of Sars-CoV-19 pandemic had a straightforward impact on the environment because of the forced stop of several human activities during the first 2020 social lockdown (Ibarra-Vega, 2020; Paital et al., 2020). Le Quéré et al. (2020) reported that CO_2 emission decreased between 11 % and 25 % by April 2020 at global level. Also, the level of particulate matter, included in the group I carcinogens by the World Health Organization (WHO, 2019) decreased drastically with the lockdown (IQAir, 2020). Several effects of the 2020 social lockdown have also been reported in the marine environment (Khan et al., 2021). The interruption of discharging industrial effluents, as well as other wastewater sources, into aquatic systems have led to an apparent increase in water quality further contributing to improved ecosystem health. Sala et al. (2022) highlighted that the level of oligotrophy significantly increased in the marine coastal area of Blanes Bay (Spain) due to several factors such as the reduction in freshwater input through treated wastewater and the reduction of fisheries and recreation activities. Coll et al. (2021) investigated the effects of the social lockdown on the fishing pressure along the Catalan coast (Spanish Mediterranean). These authors highlighted that fishing efforts during this time decreased by about 34 %, landing of seafood of about 49 % when compared with the data available for the same period of the year from 2017 to 2019.

It is likely that the positive effect of lockdown on the marine environment, as recorded almost globally, also interested the Gulf of Naples and the coast of Ischia Island. As in all the coastal areas that experienced such an unprecedented break of anthropogenic activities, one important question to be considered is "what happened once the lockdown ended and human activity resumed?" To answer this question, we evaluated the effects of the resumption of activities, particularly focusing on the temporal and quantitative trend of release of anthropogenic-discharged chemicals in seawater and their effects on coastal marine biota. For this purpose, an active biomonitoring was carried out transplanting adult mussels of the species *Mytilus galloprovincialis* in three sites of the Ischia Island (Ischia Harbour, 40°44′34″N 13°56′27″E; San Pietro shore, 40°44′47″N 13°56′40″E; Castello Aragonese, 40°43′56″N 13°57′37″E), subjected to different extent of human pressure, during a 55-day exposure after the end of the social lockdown. Ischia Harbour is characterized by intense ship traffic and boating, hosting both commercial ships and tourist boats, as well as several restaurants and hotels. The San Pietro shore is where the Ischia Marine Centre is located and the starting point of one of the underwater sewage lines of the Ischia municipality (Fig. S1) that discharges waste waters at 700 m from the shore at a depth of 55 m. The Castello Aragonese area features several restaurants and hotels and is heavily frequented by beachgoers and small tourist boats. We expected that the resumption of activities would result in a differential increase over time of chemical contamination among the 3 sites. Particularly, we expected that the harbour of Ischia, being characterized by intense ship traffic and boating, would show higher levels of contamination in respect to the other two sites. For this purpose, a selection of relevant PPCPs and PAHs were measured in the soft tissue of mussels. This study was informed by previous experience in bioaccumulation in sponges from the Maldivian coastal areas (Rizzi et al., 2020, 2023) and by the recent Beiras' review (2021), which reported the bioaccumulation and high ecological risk for a series of PPCPs, mainly the UV filters (e.g., octocrylene, 4-MBC, benzophenone-3), antibiotics (e.g., clarithromycin, erythromycin, tetracycline, azithromycin,

ampicillin, ciprofloxacin, sulfamethoxazole, norfloxacin), lipid regulator (simvastatin), the anticonvulsants carbamazepine, the antidepressant fluoxetine, and the food preservatives groups of parabens and caffeine. A suite of biomarkers related to energetic metabolism, detoxification, oxidative stress and oxidative damage was also applied to assess the potential onset of adverse effects in organisms.

2. Materials and methods

2.1. In situ translocation of M. galloprovincialis

M. galloprovincialis specimens were bought from a certified cultivation facility for human consumption in Bacoli (40°48′38.9″N, 14°05′03.0″E). The mussels were transported by a high-speed hydrofoil (one-hour journey) in cool boxes containing ice packs to keep the temperature low and to avoid further stress. Individuals meeting specified criteria (shell length 4.0 ± 0.5 cm) underwent a meticulous cleaning and selection process. Rigorous examinations ensured shell integrity, with only undamaged animals utilised for experiments, and removal of epibionts, such as barnacles and algae, during the cleaning phase. During the cleaning phases, the mussels were kept in cages in the sea area in front of the Ischia Marine Centre (Villa Dohrn), corresponding to the San Pietro site. These cages were attached to the breakwater rocks in front of the Villa. The cleaning phases lasted for one day, and the mussels were then kept for an additional day in the cages, which were already prepared for transplantation to the three sites, to allow them to recover.

The exposure of bivalves started on May 14th, 2020, coinciding with the initial resumption of coastal activities following the lockdown imposed by the Sars-CoV-19 pandemic, and lasted for 55 days until July 8th. A total of 90 individuals ($N = 45$ for chemical analysis, N = 45 for biochemical analysis) were collected from the selected batch on day 0, corresponding to T0, to serve as the reference point for the experimental starting conditions. Remaining mussels were divided and placed in submerged nets, with 100 individuals in each, at three exposure sites located at a depth of 1.20 m ± 0.1 m (Fig. 1): Harbour, San Pietro, and Castello. For each site and at each programmed sampling time, three submerged nets were utilised. Surface water temperature was continuously measured throughout the experiment using *in-situ* systems (Env-Logger by ELECTRICBLUE). It ranged from 20 °C at the beginning of the translocation to 26 °C at the end (55 days) (Fig. S2).

After 15 days (T1), 36 days (T2), 55 days (T3) of *in situ* exposure three replicates were collected from each of the 3 sites (N = 45 for chemical analysis, N = 45 for biochemical analysis each replicate). The organisms were sacrificed and divided by type of analysis (chemical, biochemical). For each experimental replicate, the 45 individuals were divided into 9 replicates made by 5 individuals pooled together. The entire soft tissues were collected for chemical analysis, while digestive glands were used for biochemical measurements. Samples were freeze and then stored at -20 °C or at -80 °C prior to chemical or biochemical analysis, respectively.

2.2. Biomarker analysis

The biomarker analyses were carried out on pooled digestive glands. Each pool was manually homogenised with a mortar. Each homogenised sample was divided in different aliquots for different biochemical assays. A suite of biomarkers was analysed including metabolic capacity (electron transport system activity, ETS), energy reserve (glycogen content, GLY), oxidative stress and detoxification (superoxide dismutase activity, SOD; catalase activity, CAT; glutathione peroxidase activity, GPx and glutathione S-transferases activity, GST) and oxidative damage (lipid peroxidation levels, LPO; protein carbonylation levels, PC). A detailed description of each method is reported in the SM.
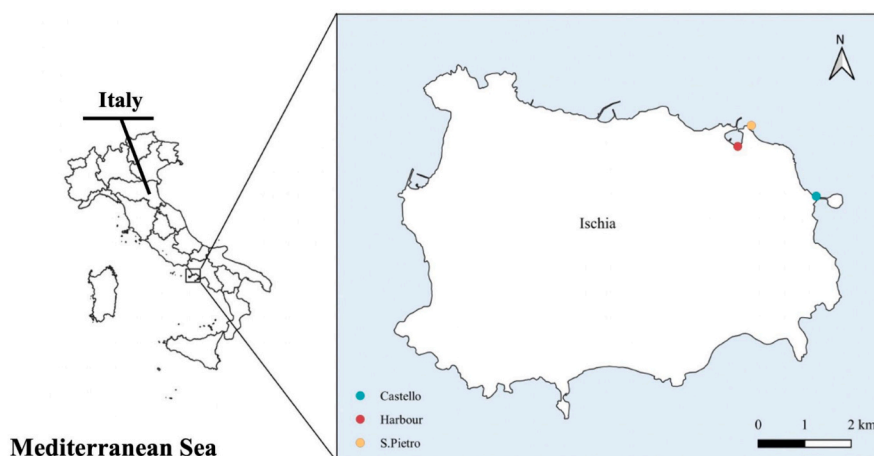
Fig. 1. Sampling sites. Map showing Ischia Island and the three sampling sites.

2.3. Chemical analysis

The contaminants selected for this study belong to different categories: caffeine (CAFF); pharmaceuticals (fluoxetine [FLX- CAS N. 54,910–89-3] and its metabolite norfluoxetine [NFLX- 30194-43-3]; carbamazepine [CBZ- CAS N. 298–46-4]; erythromycin [ERY- CAS N. 114–07-8]; diclofenac [DCL- CAS N. 15,307–86-5]; paracetamol [PAR- CAS N. 103–90-2]; sildenafil [SILD- CAS N. 171,599–83-0]; tadalafil [TAD- CAS N. 171,596–29-5]); preservatives (methylparaben [MP- CAS N. 99–76-3]); artificial sweetener (sucralose [SUCR- CAS N. 56,038–13-2]); UV filters (3-(4-methylbenzylidene) camphor [4MBC- CAS N. 36,861–47-9]; benzophenone-1 [BP1- CAS N. 131–56-6]; benzophenone-3 [BP3- CAS N. 131–57-7]); polycyclic aromatic hydrocarbons (naphthalene [Nap- CAS N. 91–20-3]; acenaphthylene [Acy- CAS N. 208–96-8]; acenaphthene, [Ace- CAS N. 83–32-9]; fluorene [Flu- CAS N. 86–73-7]; phenanthrene [Phen- CAS N. 85–01-8]; anthracene [Ant- CAS N. 120–12-7]; fluoranthene [Fl- CAS N. 206–44-0]; pyrene [Pyr- CAS N. 129–00-0]; benzo[*a*]anthracene [BaA- CAS N. 1718-53-2]; chrysene [Chr- CAS N. 218–01-9]; benzo[*b*]fluoranthene [BbF- CAS N. 205–99-2]; benzo[*k*]fluoranthene [BkF- CAS N. 207–08-9]; benzo[*a*] pyrene [BaP- CAS N. 50–32-8]; dibenz[*a,h*]anthracene [DBahA- CAS N. 53–70-3]; indeno[1,2,3-*cd*]pyrene [IP- CAS N. 193–39-5]; benzo[*ghi*] perylene, [BghiP- CAS N. 191–24-2]). The chemical analyses were conducted on pooled soft tissues that were freeze-dried and subsequently homogenised with a mortar. Each sample was divided in aliquots for different chemical analyses. The extraction was achieved by microwave-assisted extraction (MAE). The identification and quantification of the selected compounds was performed by gas chromatography (GC) - mass spectrometry (MS) or ultra-performance liquid chromatography (UPLC) - mass spectrometry. Details on the methods are described in SM.

2.4. Statistical analysis

Univariate and multivariate analyses were carried out to test for differences in biomarker and chemical results among sites and sampling times. The experimental design included two crossed factors: site, fixed with three levels (Harbour, San Pietro, Castello), and time, random, with four levels (T0, T1, T2, T3). Analysis of variance (ANOVA) were carried out independently per each biomarker and chemical considered using the PERMANOVA routine on the Euclidean distance matrix calculated per each variable. A non-parametric PERMutational multivariate ANalysis Of VARiance (PERMANOVA) (Anderson, 2001) was performed to assess multivariate differences among sites and sampling times using the whole dataset comprehensive of biomarkers and chemical contaminants. The test was carried out on the Bray Curtis similarity matrix of standardised data and visualised using Principal Coordinates Analysis (PCO). The univariate and multivariate analyses were carried out using the software package PRIMER v7 (Plymouth Routines in Multivariate Ecological research).

3. Results

Measurements of chemical contaminants and biomarkers of mussels collected at T0 are shown in Table S1 and S2. The PERMANOVA main test considering all variables from chemical and biomarkers analyses showed a significant effect of the interaction 'site' x 'time' ($p = 0.0001$ $F_{6,35} = 8.9241$) as well as of both the single factors (site: $p = 0.0276$ $F_{2,35} = 3.9689$; time: $p = 0.0001$ $F_{3,35} = 28.622$).

3.1. Accumulation of contaminants

Among the contaminants analysed, only CAFF, CBZ, ERY and the 15 PAHs were quantified over the limit of detection (LOD) in soft tissues. The BP-1 resulted in concentration between the limit of detection (LOD) and the limit of quantification (LOQ). All other contaminants were below the LOD. The statistical analysis on the levels of contaminants accumulated in mussel soft tissues showed a significant effect of the interaction between site and time as well as of both the single factors (Table S3). A huge variability in the accumulation of the 15 PAHs emerged, so that no statistically significant differences were observed between the three sites and over time. Anyway, a peak of accumulation occurred at the Harbour site at T1, followed by a sharp decline over time (Fig. 2). Regarding CAFF, a significant difference has been observed for the factors 'site', 'time' and their interaction (Table S3). CAFF in mussels located at the Harbour was always higher than the level at T0 (Fig. 2). Mussels from San Pietro accumulated less CAFF compared to the other sites approaching the level measured at T0. The highest levels of CAFF were measured in soft tissues of mussels translocated at Castello, up to ten times above the levels of T0 at T1 and T2. Measurement of ERY displayed significant difference between the factor 'time' and the interaction 'site x time' (Table S3). Indeed, a similar extent of accumulation was measured in the three sites, always above the accumulation measured at T0 (Fig. 2). In mussels from the Harbour a higher accumulation was observed at T1, followed by a significant reduction at later time. In mussels translocated at San Pietro a U-shape profile of accumulation was observed, with a significant reduction occurring at T2. Conversely, in mussels from the Castello a bell-shape profile of accumulation was observed peaking at T2. As for the accumulation of CBZ, significant differences for the factors 'site', 'time' and their interaction were obtained (Table S3). Any accumulation occurred in mussels translocated at San Pietro, while a similar profile was observed in
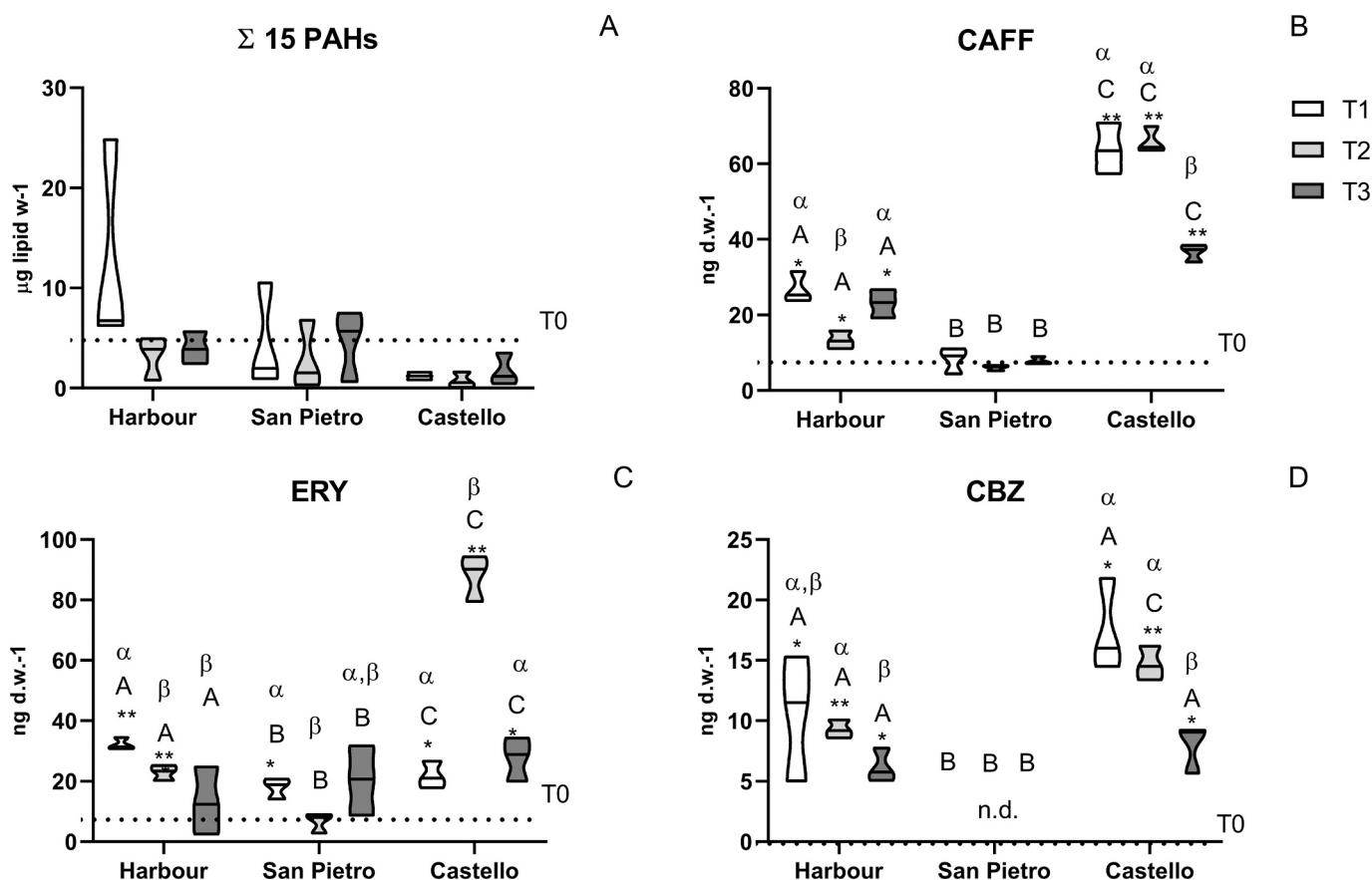
Fig. 2. Accumulation of contaminants. Mean values ± standard deviation of the levels of PAHs (A), CAFF (B), ERY (C) and CBZ (D) in soft tissues of *M. galloprovincialis* collected at the three sites at T1, T2, T3. T0 is shown as a continuous line. N.d. means not detected. The presence of one, or two asterisks (*/**) indicates a statistically significant difference in relation to T0 ($p \leq 0.05/p \leq 0.001$). The capital letter indicates a statistically significant difference between different sites within the same time level, the Greek letter between different times within the same site level.

mussels from Harbour and Castello, where a higher accumulation occurred at T1 followed by a decrease thereafter (Fig. 2). Finally, BP-1 was detected only at T1 in mussels translocated at Harbour, whereas at San Pietro, traces of BP-1 were detected only at T3 (Table S3). Conversely, in mussels from Castello, traces of BP-1 (with concentration between LOD and LOQ) were observed at all times of exposure in situ.

3.2. Biomarkers

The integration of all biomarkers of energy metabolism, oxidative stress and oxidative damage showed a significant effect of the single factors and of their interaction (Table S4).

3.2.1. Antioxidant/detoxification enzymes

The activity of SOD, CAT and GPx was measured to assess the potential onset of oxidative stress. The GST was analysed, since it is a key enzyme involved in detoxification of toxic chemicals (Regoli et al., 2011). Regarding SOD enzyme, significant difference was observed for the interaction 'site × time' (Table S4). The activity in mussels translocated at the Harbour was significantly lower than T0 at T1 and T2 and returned to basal levels at T3 (Fig. 3). In mussels from San Pietro, the level of SOD activity increased at T1, being significantly higher than the other sites, followed by a decrease close to T0 levels already at T2. Any modulation of SOD activity has been observed in mussels translocated at Castello over time. The activity was lower than T0, significantly only at T3.

As for the CAT enzyme a significant difference for the 'site × time' interaction was obtained (Table S4). Mussels translocated at the Harbour showed significantly lower activities than those measured at T0

and in the other two sites (Fig. 3). In mussels from San Pietro a time-dependent decrease of CAT activity was observed. Conversely, the organisms from Castello showed a bell-shaped profile of CAT activity, peaking at T2, and decreasing significantly at T3.

Regarding GPx activity, the PERMANOVA analysis reported a significant difference for the interaction 'site × time' (Table S4). The profile of this enzyme was similar in mussels translocated at the Harbour and Castello, with no differences in the activity over time, except for T3 at Castello, which was significantly lower than T0 (Fig. 3). Conversely, mussels from San Pietro showed an increase of GPx activity over time, with T2 and T3 significantly higher than T0.

Regarding the GST activity, the interaction 'site × time' was statistically significant (Table S4). In mussels from the Harbour, the GST activity at T1 and T2 was lower than T0 but increased later of translocation (Fig. 3). In mussels from San Pietro, a significant increase of GST activity with respect to T0 was observed at T1 and T2, followed by a decrease at T3, approaching the levels of T0. Any modulation of GST has been observed in mussels from Castello over time, with activities always lower than T0.

3.2.2. Energy metabolism

ETS activity and GLY content were measured as marker of metabolic performance. The ETS activity showed a statistically significant difference for the factor site and time and their interaction (Table S4). In mussels translocated at the Harbour, there was a steady increase in ETS activity from T1 to T3, initially significant from T1 to T2, with values statistically different from T0 at later time (Fig. 4). The ETS profile at San Pietro differed from the other two sites, since here the ETS activity profile showed a significant decrease between T1 and T2, followed by
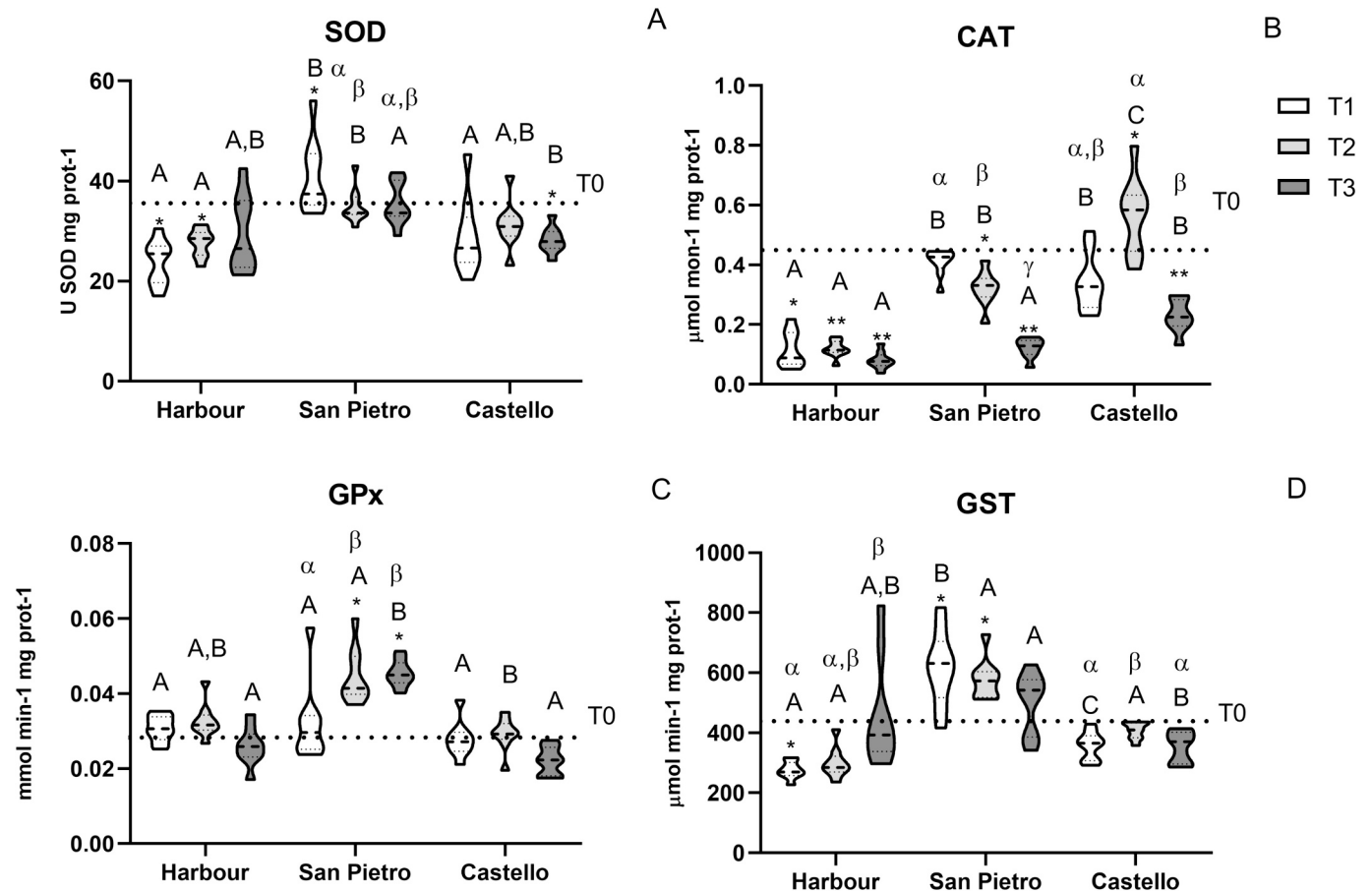
Fig. 3. Oxidative stress. Mean values ± standard deviation of SOD (A), CAT (B), GPx (C) and GST (D) in *M. galloprovincialis* collected at the three sites at T1, T2, T3. T0 is shown as a continuous line. The presence of one, or two asterisks (*/**) indicates a statistically significant difference in relation to T0 ($p \leq 0.05/p \leq 0.001$). The capital letter indicates a statistically significant difference between different sites within the same time level, the Greek letter between different times within the same site level.

significant increase between T2 and T3. The ETS activity in mussels from Castello was higher than those measured in the other sites. A slight decrease of activity over time was observed, anyhow, levels were always significantly higher than T0.

As for GLY content, a statistically significant difference for the interaction 'site × time' was observed (Table S4). Mussels translocated at the Harbour showed a GLY content always higher than T0 (Fig. 4). Also, in mussels from San Pietro the GLY content was higher than T0 with significant differences at T1. The GLY levels were on average higher in mussels translocated at Castello in comparison with the other sites.

3.2.3. Oxidative damage

The levels of LPO and PC were measured as an indicator of the possible damage to lipids and proteins. For LPO, the PERMANOVA analysis showed a statistically significant difference for the interaction 'site × time', but not for the factor site (Table S4). In mussels from the Harbour, LPO values were kept constant and significantly lower than T0. Organisms from San Pietro showed a bell-shaped profile of LPO levels, with a significant increase from T1 to T2, followed by a significant decrease from T2 to T3. LPO levels in organisms from Castello were on average higher than at the other sites and higher than T0. In this site, the profile of LPO reached a maximum peak at T3 (Fig. 4).

Regarding PC, the PERMANOVA analysis reported significant differences for the factor time, interaction 'site × time', but not for the factor site (Table S4). In organisms from the Harbour, the PC levels were lower than T0 at all times of translocation (Fig. 4). In mussels from San Pietro, a significant increase of PC content was observed at T1 with respect to T0, also higher than the levels measured in the other sites,

followed by a decrease at later times of translocation. In organisms from Castello the levels of PC were always similar to T0.

3.3. Multivariate analysis

The PCO analysis was carried out to visualize the similarity among sites during the four sampling times and their relationship with pollutants and biomarkers (Fig. 5). PCO1 and PCO2 explained 75.9 % and 12.7 % of the total observed variation, respectively. At a general level (considering all times and both analyses), the three sites were clearly separated from each other, and the distribution was different: for the San Pietro site group T2 and T3 appeared to be overlapped with T0, while T1 was separated from the other time points on PCO2. This separation is not as significant as on the PCO1 axis, since PCO2 only explains 12.7 % of the variation. The Harbour site groups constituted another cluster, in which T2 resulted separated from the other two times and tightly clustered. The Castello site showed T1, T2 and T3 less clustered each other and separated from the other sites. High Molecular Weight PAH (HMW_PAH; 4–6 rings) appeared as the most relevant variables related to the spatial separation of the Harbour group, while for the Castello group the accumulation of PPCPs, LPO and biomarkers of energy metabolism resulted more relevant. The spatial separation of San Pietro appeared largely related to biomarkers of oxidative stress (SOD, GST, GPx) and PC.

4. Discussion

The objective of this study was to assess the pattern of a suite of
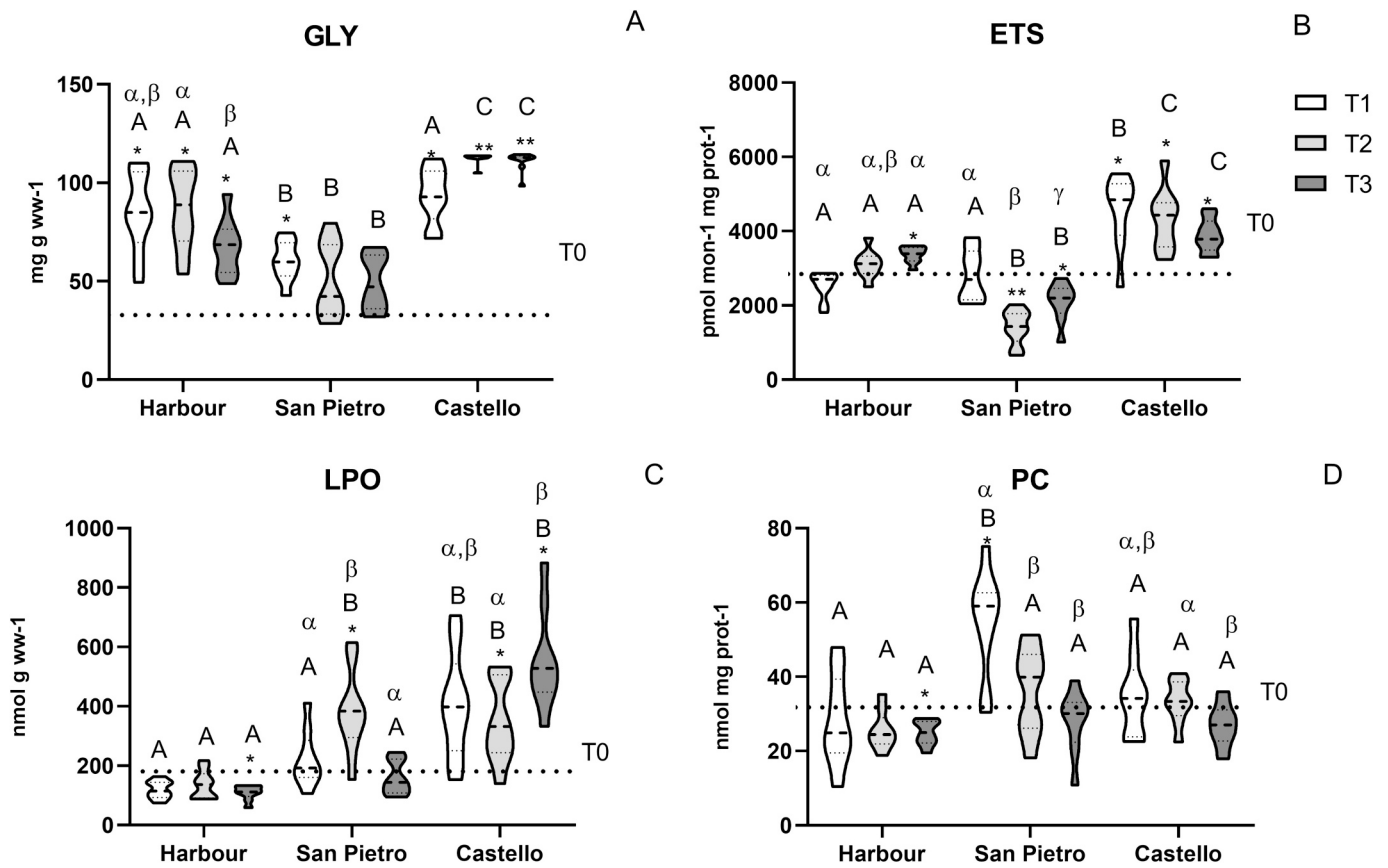
Fig. 4. Energy metabolism and oxidative damage. Mean values ± standard deviation of ETS (A), GLY (B), LPO (C) and PC (D) in *M. galloprovincialis* collected at the three sites at T1, T2, T3. T0 is shown as a continuous line. The presence of one, or two asterisks (*/**) indicates a statistically significant difference in relation to T0 ($p \leq 0.05/p \leq 0.001$). The capital letter indicates a statistically significant difference between different sites within the same time level, the Greek letter between different times within the same site level.
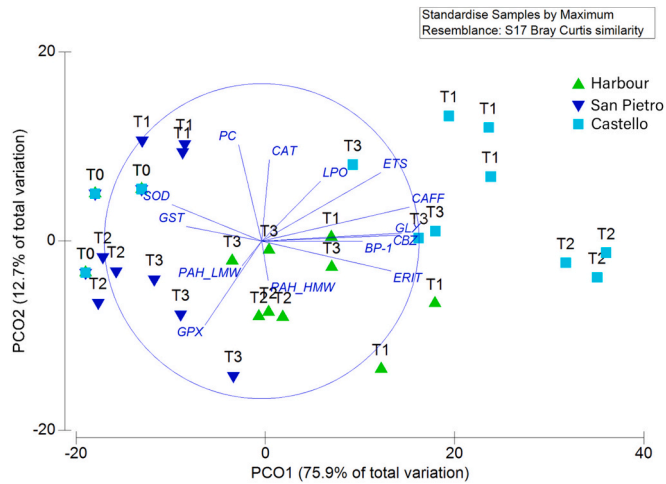

Fig. 5. PCO showing the similarity among sites during the four sampling times. The correlation with contaminants' concentrations and biomarkers with sites during time is also reported. The circle corresponds to the maximum possible vector's length along any direction.

biomarkers (energy metabolism and oxidative stress) over time and site, linking the effects with the bioaccumulation trend of anthropogenic contaminants in areas of various human activities. To this aim, an active biomonitoring has been carried out on the Ischia Island using the Mediterranean mussel *M. galloprovincialis*, following the resumption of anthropic activities on the island after the end of restrictions put in place to prevent the spread of the Sars-CoV-19 pandemic in 2020.

The levels of PPCPs detected in mussels are comparable to what has been reported in other marine coastal areas. For instance, CAFF accumulation in *Mytilus* spp. along the California coast (USA) ranged from 19 to 68 ng g^{-1} dry weight (d.w.) (very close to those found in individuals from the Castello site) (Maruya et al., 2014) and up to 35.5 ng g^{-1} d.w. in sponges from Maldivian Islands (Rizzi et al., 2020, 2023). Regarding CBZ, the accumulation in marine bivalves collected in several marine coastal areas from the Mediterranean Sea and the Atlantic Ocean has been reported (Almeida et al., 2021), with levels up to 13 ng g^{-1} d.w., close to the one measured in mussels from the Harbour and Castello at T1 and T2.

The accumulation of BP-1 in marine organisms has been seldom monitored, and most of the studies reported concentration below the limit of detection (Castro et al., 2022), as observed in our study, even though in mussels from the European region BP-1 has been detected with levels up to 94 ng g^{-1} d.w. (Cunha et al., 2018). To the best of our knowledge, no information are currently available on the accumulation of ERY in natural bivalve populations. Therefore, our study contributes to fill the current gap of knowledge on the availability of both BP-1 and ERY for marine organisms. Results will be useful to assess the environmental risk of these chemicals for benthic communities.

The profile of contamination of the contaminants detected in mussels did not show an increase over time, but rather a reduction of the accumulation with increasing translocation period, in the Harbour and Castello sites. This suggests that the gradual recovery of activities and of the resident population on the island following the Sars-Cov-2 lockdown has not led to an increase in the introduction of PAHs and PPCPs into the coastal system. The only exception is the peak of ERY observed

at T2 at Castello, which could be linked to a point-source release in the area. It is reasonable to posit that the environmental pseudo-persistence, as defined by Daughton and Ternes (1999), in these areas is related to human consumption, as ERY is an antibiotic drug used to treat a range of diseases, including those of the respiratory tract. Although this hypothesis cannot be substantiated without epidemiological data, it is plausible to suggest that it was taken to combat bacterial infections, which were particularly common after SARS-CoV-2 infection.

Based on the profile of accumulation of contaminants, we can assume that the chemicals accumulated by the mussels during the translocation reflect the background of the island and they are not affected by the intensification of human activity in relation to the tourist season, even though some transient peaks of release could occur, as seen for ERY. Nevertheless, conducting further analyses over an extended period of time would confirm our observations.

Regarding biological variables, as for the metabolic performance, in Harbour and Castello sites ETS activity increased with respect to T0, suggesting that the organisms have activated metabolic defence reactions. This does not occur at the expense of the GLY energy resource. Conversely, in San Pietro ETS activity decreases over time. Given the key role of ETS activity in the production of energy to generate ATP (Cammen et al., 1990), the parameters of energy metabolism suggest that organisms are in a condition of homeostatic compensatory response, with an increasing amount of energy to boost the machinery for cellular protection and to maintain basal functions (Sokolova et al., 2012). However, this condition may entail a trade-off towards other important functions such as growth and reproduction and in the long term may progress to a condition of severe stress (Sokolova et al., 2012).

Also, the profile of oxidative stress/damage biomarkers showed a clear separation of the three sites. In organisms from the Harbour, we generally observed lower values of enzymatic activity compared to the other sites and to T0, with a significant reduction of CAT activity over time. A depletion of antioxidant enzymes has been often reported in organisms subjected to long-term exposure to pollutants both in laboratory conditions and in the field (Regoli et al., 2011). Thus, the significantly lower levels of LPO and PC measured in mussels compared to T0 and to other sites suggest that the antioxidant efficiency of the organisms is not compromised since other compensatory mechanisms are active to prevent the onset of oxidative damages.

In mussels from San Pietro, we observed an induction of some antioxidant enzymes (GPx and GST). This suggests that even low levels of contaminants might trigger a mild pro-oxidant condition. This is also reflected in the onset of oxidative damage, such as LPO and PC, which however is transient, by virtue of the activation of antioxidant enzymes, which in the long term can counteract the stress generated and prevent the persistence of oxidative damages. Finally, in mussels from Castello, any modulation of antioxidant enzymes occurred - apart from CAT at T2 - nonetheless the onset of significant oxidative damage to lipids was observed, which persisted over time. This suggests that in the organisms from this site the antioxidant system have not been able to counteract efficiently the oxidative stress condition they faced.

Even though it is not possible to establish a cause-effect correlation among contaminants and the modulation of biomarkers, it is likely that the biological effects observed are due to the presence of toxic chemicals in the water column, since oxidative stress and damages are among the adverse effects ascribed to PAHs and the PPCPs detected (Cruz et al., 2016; Liang et al., 2020; Almeida et al., 2021; Vieira et al., 2022). The investigation of biological effects is also able to integrate potential additive or synergistic effects that could be established among the mixture of contaminants present in the water column, including chemicals below the limit of quantification or pollutants that were not measured in this study, such as metals. Natural environmental factors, such as increasing water temperature and variation in food availability, might also have influenced the temporal trend of biomarkers. For instance, a seasonal variation in the activity of ETS has been described in different aquatic organisms with an increase of activity observed during summer with respect to other seasons (Cammen et al., 1990; Fanslow et al., 2001). Similarly, increasing seawater temperature and intensity of light irradiance which occurs during summer are known to produce pro-oxidant conditions in mussels that affect the activity of antioxidant enzymes (Bocchetti et al., 2008).

The integration of chemical contamination and biomarker responses showed a different impact on the three sites as clearly evidenced by the PCO analyses: at the Harbour, as expected, we observed the highest level of PAHs, but also a considerable bioavailability of some PPCPs. The San Pietro site emerged as the least impacted by chemical pollution as highlighted by the PCO analyses where is overlapped with the results from the T0. Conversely, the Castello site proved to be the most affected by PPCPs release in the marine environment. Furthermore, the PCO highlighted how in this site a point source release of contaminants must have occurred at T2. The difference among the three sites is likely due to the higher level of touristic appeal of the village of Ischia Ponte, where the Castello site is located, compared to San Pietro and the Harbour. In Ischia Ponte, the significant presence of hotels and restaurants, coupled with the absence of wastewater treatment plants in this area, characterized solely by the existence of underwater sewage lines, may have exacerbated the presence of chemicals compared to the other two sites.

5. Conclusions

Our study contributes to increasing the current knowledge on the occurrence and availability of PPCPs in Mediterranean coastal areas, as well as their potential impact on biological resources. While our results do not enable us to establish a direct cause-effect relationship between contaminants and the biomarker response—given the potential contribution of other toxic chemicals and environmental factors like temperature—they do provide valuable insights. The observed modulation of biomarkers serves as an early warning signal for potential adverse consequences affecting essential physiological and ecological functions in the long term. The data presented in this study demonstrated the feasibility of utilising active biomonitoring as a valuable tool for planning future management activities aimed at reducing environmental pollution, with the goal of preserving biodiversity and maintaining the integrity of the ecological functions of the coastal ecosystems especially within Marine Protected Areas.

CRediT authorship contribution statement

Camilla Della Torre: Writing – original draft, Supervision, Data curation, Conceptualization. **Sara Villa:** Writing – original draft, Supervision, Data curation. **Antonia Chiarore:** Writing – review & editing, Resources, Formal analysis. **Antonio Cannavacciuolo:** Writing – review & editing, Resources. **Cristiana Rizzi:** Writing – review & editing, Formal analysis. **Luigi Musco:** Writing – review & editing, Funding acquisition, Conceptualization. **Lara Nigro:** Writing – review & editing, Formal analysis. **Marco Munari:** Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.116533.

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