A Weight of Evidence approach to support the assessment of the quality of Manila clam farming sites in a coastal lagoon

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Abstract

Aquaculture productivity in coastal lagoons is endangered by a complex interplay of anthropogenic and environmental factors, amplified by the effects of climate change in these sensitive areas.

To reach a more comprehensive assessment of farming sites quality, a quantitative Weight of Evidence approach (QWoE) is applied for the first time to data collected at four Manila clam (R. philippinarum) farming sites in the Venice lagoon (Italy). This included sediment quality, chemical bioaccumulation, and biological responses.

Results revealed a greater hazard for sites closer to the open sea. In these areas, the combination of sediment characteristics and a higher frequency of salinity and temperature stress could explain the alterations measured at a transcriptional and biomarker level. The findings demonstrate that a QWoE approach that integrates multiple sources of evidence should also include physicochemical conditions in order to better understand the impacts of human activities and other stressors on clam aquaculture productivity.

1. Introduction

Tidal wetlands, especially in temperate regions, have provided the right conditions for the development of aquaculture contributing to the economic and social growth of coastal populations. However, coastal lagoons that endured through time present traits of extensive modification (Kirwan and Megonigal, 2013), which makes them more vulnerable to impacts associated with climate change (Kirezci et al., 2020). Understanding how these anthropogenic and climate-related changes impact aquaculture farming is of foremost importance considering the fact that aquaculture production is expected to supply 65% of world seafood consumption by 2030 (Ghezzo et al., 2018).

With regard to clams production, farming sites are experiencing a decrease with mass mortality outbreaks documented worldwide (Soon and Zheng, 2019). Particularly in the last two decades, a decline in clam production has been documented in several European countries (EUMOFA, 2022), such as Portugal (Cravo et al., 2012), Italy (Milan et al., 2023), and France (Smits et al., 2020), but also in the tidal flats of Korean west coast (Nam et al., 2018) and Japan (Tezuka et al., 2012; Kozuki et al., 2013). If environmental variables can influence clams’ physiological processes (Maynou et al., 2020), it is also true that sediment plays an important role as a possible source of contaminants and/or nutrients for these bottom-feeding bivalves (Sfriso et al., 2014; Coughlan et al., 2002). However, the majority of the literature available to date has tried to understand how changes in the main water parameters (e.g., temperature, pH, salinity, chlorophyll) could influence biotic indicators or impact bivalve growth (Pastres et al., 2001; Silva et al., 2011; Tan et al., 2022). The role played by sediment is still an overlooked aspect of bivalve research.

Being at the interface between terrestrial and marine ecosystems,
coastal wetlands present the right condition for clam farming, but they are also very dynamic systems where sediment quality is influenced by multiple factors (e.g., the exchange with the open sea, contamination from the drainage basin, anthropogenic activities). With so many variables at play, focusing on results from a single type of analysis, index, or parameter might lead to a misinterpretation of evidence (Cravo et al., 2012; Soon and Ransangan, 2019). Only interdisciplinary studies can provide indications for sustainable management solutions that would limit stresses on farmed animals (Marinov et al., 2007).

With the interest of reaching a comprehensive assessment of factors influencing aquaculture productivity (Vethaak et al., 2017), a quantitative Weight of Evidence (QWoE) approach represents a suitable way to collect and assemble multiple sources of data (Suter et al., 2017), including chemical exposure and biological effect data (Regoli et al., 2019). WoE approaches have been applied to aquaculture before, but mainly with the purpose of assessing the ecological impact originating from aquaculture production (Silva et al., 2016; Zhang et al., 2020). Here, a structured and transparent approach is instead chosen with the exploratory intent of achieving a more comprehensive assessment of the quality of clam farming sites in transitional environments.

The Venice Lagoon in Italy is used in this study as a representative example for the application of the integrated approach. This lagoon is the most productive areas of edible clams (Ruditapes philippinarum) in Italy (STECF-22-17, 2023) but it has also experienced a drastic decrease in production in the last decade (Milan et al., 2023). To elucidate the quality of farmed areas, data on sediment quality and organism biological responses were collected in a two-year experimental campaign at four Manila clam farming sites. For two seasonal campaigns, that took place across the first activations of a new storm-surge barrier control system (called MoSE), data were funneled into four Lines of Evidence (LoEs) representing evidence from (i) chemical characterization of surficial sediment, (ii) bioaccumulation of contaminants in clams' soft tissues, (iii) biochemical biomarkers indicative of oxidative stress and neurotoxicity, and (iv) transcriptomic alterations. The latest is introduced here for the first time in a quantitative WoE application to in-situ assessments allowing gene expression profiling to constitute an additional line of evidence (Cecchetto et al., 2023). Incorporation of physicochemical data measured with multiparametric probes contributed to a proper interpretation of biological responses observed in clams. The integrated approach provided an exploratory tool for the evaluation of clams farming sites that can be extended to other farming areas worldwide.

2. Materials and methods

2.1. Study area

About the 13% of the global coastal area consists of lagoons (Scarpa et al., 2022), with the Venice lagoon representing the largest transitional ecosystem in the Mediterranean (Madricardo et al., 2019). This complex maze of channels and salt marshes is connected to the Adriatic Sea by three inlets through which water and sediment are exchanged (Fig. 1A). Since October 2020, the three inlets are regulated by a system of storm-surge barriers named MoSE to prevent the flooding of the Venice historic centre. As sea levels rise (Kirezci et al., 2020), the operation of MoSE (which started in October 2020) is expected to increase in frequency and in the duration of closures, potentially influencing physicochemical parameters and sediment quality and resuspension in the lagoon (Tognin et al., 2022).
2.2. Experimental campaigns

Two experimental campaigns (May 2019–May 2020, 1st year; May 2020–May 2021, 2nd year) were carried out in pre-existing licensed farming areas of Manila clam (R. philippinarum) located in the southern basin of the Venice lagoon (Fig. 1A and B). For both monitoring years, 5000 spats of Manila clam, supplied by Satmar (France), were seeded (in approximately 10 m²) in May 2019 (1st year) and in May 2020 (2nd year) in farming areas at increasing distances from the Chioggia inlet. Investigated farming sites are named 1F, 2F, 3F, and 4F following the progressive distance from the lagoon inlet (Fig. 1B). Both the first (May 2019–May 2020) and the second year (May 2020–May 2021) were interested by four seasonal samplings of surficial sediment for chemical characterization (Fig. 1C). After annual seeding in May, animals were sampled seasonally in correspondence with sediment collection, to assess bioaccumulation of pollutants, cellular biomarker alteration, changes in gene expression profile, and the physiological Condition Index, i.e. CI (measured as the ratio of soft tissue dry weight over shell dry weight). CI values are reported in Supplementary material SM1. About 300 clams were randomly collected in each site and in each sampling time using a manual rake.

Of all the samplings collected over the two-year study (Fig. 1C), this work focuses on May 2020 and May 2021. Focusing on May samplings, which are at the end of both sampling campaigns and corresponded to the reaching of commercial size in clams, allowed to have a track record of chemical, biological, and physicochemical information spanning over several months. This helped contextualize within a wider timeframe both chemical and biological data collected in May whereby four LoEs are formed and successively weighed in a WoE approach. The choice of this campaign-time was also based on the possibility to investigate the quality status of sites before and after the activation of the MoSE barriers, which took place between October 2020 and February 2021 (Fig. 1C). Based on previous knowledge of its environmental quality and the past good productivity (Milan et al., 2023), site 3F represented the ambient condition used as reference for bioaccumulation, biomarkers, and transcriptomics analyses.

In addition to biota and sediment sampling, two multiparametric probes were located in proximity to sites 1F and 2F (Probe North) and to site 4F (Probe South), respectively (Fig. 1B). Probes recorded water temperature (°C) and salinity (%) continuously from July 2019 to May 2021. The measurements were used to attain a better interpretation of the biological response of clams. Table S6 in Supplementary material SM1 reports, for brevity, the monthly averaged values of temperature and salinity recorded by both probes.

2.3. Chemical characterization

Chemical characterization in the two campaigns included analyses of contaminants in surficial sediment that was collected at each farming site using a grab sampler. Chemical analysis included trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). All the details on sample treatment and analytical procedures are given in Milan et al. (2023) and briefly summarized in Supplementary material (SM1) while SM2 reports the measured concentrations of the analysed pollutants.

Concentrations of pollutants were used to calculate sediment chemical hazard by following the approach proposed by Piva et al. (2011), which, in recent years, has been largely validated in numerous case studies for the environmental risk assessment of dredged sediment, marine incidents and constructions, or emerging contaminants (Benedetti et al., 2012; Lucia et al., 2023; Regoli et al., 2014, 2019). The approach consists of a comparison of measured concentrations with sediment environmental quality standards (EQSs). For this case study, EQSs were set according to the national Legislative Decree 172/2015, but they can be easily adapted to the local or national context of interest.

The calculation of the hazard index, HI_s, whose full procedure is detailed elsewhere (Piva et al., 2011), is completed with the attribution of a hazard class (namely absent, slight, moderate, major and severe) to the sediment sample.

2.4. Bioaccumulation in Manila clams

For bioaccumulation analyses, soft tissues (whole body) of clams were collected and pooled to form composite samples (about 60 individuals for metals and 100 individuals for organic pollutants). Chemical analyses were performed to determine the concentrations of PCDD/Fs, PCBs, PAHs, and trace elements. Details of sample treatment and analytical procedures are given in Supplementary Material (SM1), while concentrations are presented in SM2.

Concentrations in clams’ soft tissues contributed to assess potential bioavailability of pollutants by forming a second LoE, which follows the approach presented by Regoli et al. (2019). The variation in concentration between clams from sites 1F, 2F, and 4F and that of clams from the ambient site 3F is calculated. Depending on the ratio, one of the five classes of bioaccumulation effect, from absent to severe, is assigned to each contaminant, and the hazard index, HI ba, is the result of the distribution of analysed parameters within the different classes of effects.

2.5. Biomarkers analysis

Antioxidant enzyme activities measured in gills and digestive gland included superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR). The detoxifying activity of glutathione-S-transferase (GST) in the digestive gland was used as a proxy of contaminants presence in the environment. Measurement of the activity of acetylcholinesterase (AChE) was used as a biomarker of neurotoxicity in gills. Detailed analytical procedures, statistical analysis information, and oxidative stress, neurotoxicity, and detoxification biomarkers evaluated in clams are presented in the Supplementary material (SM1).

Results from biomarker analyses were used to calculate the hazard index associated to the third LoE. According to the methodology proposed in Piva et al. (2011) and Regoli et al. (2019), biomarkers variation measured in organisms when compared to the ambient sample (organisms from site 3F) is evaluated based on the relevance of the biological endpoint in the light of current mechanistic knowledge, and a threshold for changes of biological significance. Such variation is then corrected by a function Z to account for its statistical significance, which in this study is calculated by means of a nonparametric Mann-Whitney test, performed in R Core Team (2023). The distribution of biomarkers within five classes of effect (from absent to severe) contributes to the derivation of the hazard index, HI bm, and relative class of hazard.

2.6. Transcriptomics analysis

Transcriptomics analyses investigated changes at a molecular scale by studying gene expression profiles (RNA-seq) in clams collected from the four farming sites. Similar to the biomarker analysis, organisms reared in site 3F were used to elicit transcriptional alterations in clams from 1F, 2F, and 4F.

Results from the Differential Expression Analysis were used to perform Gene Set Enrichment Analysis, GSEA (Subramanian et al., 2005), on the Hallmark database to reveal pathways that were significantly altered. Results are presented in Supplementary material SM1, where data analysis and sample treatment are also detailed.

The line of evidence that translates analysis of transcriptional alterations into a hazard index suitable to be incorporated into a WoE approach is presented in Cecchetto et al. (2023). Briefly, gene sets, whose different expression was deemed statistically significant, were organized into eight higher biological categories. The level of deregulation in gene sets and the biological importance of such alteration contribute to assess if the transcriptional effect endured by the
category is either absent, slight, moderate, major or severe. Depending on the distribution of categories within the five classes of effect, the hazard index, HI, is calculated and a class of hazard assigned.

2.7. LoEs integration and weighing

Chemical characterization of sediment and biota along with cellular and molecular results were grouped into four distinct LoEs (chemistry, bioaccumulation, biomarker and transcriptomics) that were eventually integrated and weighed, by first normalizing the hazard indexes to a common scale (from 0 to 100). Data quality, properties and resolution (i.e., metadata) contributed to evaluate to which extent the conclusions reached by single LoEs were reliable and relevant to assess the quality status of the farming sites (Bates et al., 2018). The normalized hazard indexes were thereby multiplied by specific scores, \( w_i \), that quantify the evidence strength. The scoring system followed the rating presented by Bates et al. (2018). Based on this rating, the expert qualitative assessment of evidence metadata was transformed into a strength score, ranging from 0 (no strength) to 1 (perfect strength). In this study, a lower weighting was given to the evidence from bioaccumulation (\( w_{ba} = 0.7 \)), biomarker (\( w_{bm} = 0.7 \)) and transcriptome (\( w_{tc} = 0.5 \)) analyses compared to weights assigned to chemistry data (\( w_c = 0.9 \)). Bioaccumulation evidence was based on clams, but concentrations in clams’ soft tissues are not always representative of environmental exposure (Ademollo et al., 2017; Moschino et al., 2012). Because accumulation in clams should be cautiously interpreted, a weight of 0.7 indicating medium to high strength was preferred for this evidence. An equal weight of 0.7 was assigned to the biomaker LoE since this evidence was constructed on a limited number of biomarkers for which threshold values are available. The weight for the LoE transcriptomics was further lowered with respect to other LoEs because transcriptional changes may be decoupled from protein levels/activities. Chemistry was the most influential evidence given the fact that its results are independent of the ambient site. However, the calculation of the chemical hazard index includes only the regulated contaminants leaving aside those of emerging concern for which EQSs are not yet defined in the national regulations. To account for this limitation but, at the same time, to enhance the independence of chemistry on the ambient site, a weight of 0.9 was deemed appropriate. Scores were then used to calculate the WoE hazard index, \( HI_{WoE} \), as follows:

\[
HI_{WoE} = \frac{\sum_{i=1}^{N} w_i HI_i}{\sum w_i}
\] (1)

A class of hazard is finally assigned to the overall weighted value following the classes reported above.

3. Results and discussion

3.1. Chemical characterization

Total concentrations of trace elements in 1F were similar in the two sampling campaigns, except for an increase in As (Supplementary material SM2). For site 2F, slight temporal variation characterized Cr concentration, while in 3F As and Cd presented the greatest variation in time. A wider temporal variability was observed in 4F, with the exception of Cu, Pb, and Hg. Concentrations showed higher values for most elements at sites 2F and 4F, which may be ascribed to the greater percentage of fine particles in sediments, compared to site 1F, which is mostly sandy (Milan et al., 2023). Sediments from site 3F have in general concentrations comparable to those found at site 1F, except for Cr and Ni - elements with a prevalent lithogenic origin – whose total concentrations were similar to those observed at sites 2F and 4F. Higher Cr concentration levels have been previously reported for the south-western part of the Venice lagoon in comparison to the other basins (Apitz et al., 2007; Zonta et al., 2018). Overall, trace element concentrations measured in this study are within the ranges reported for the study area and its surroundings (Zonta et al., 2018; Moschino et al., 2012).

The ambient site 3F showed the lowest levels of organic pollutants. Site 1F presented a marked temporal variability for PCDD/Fs and PAHs, with significantly higher values in sediment collected in the first monitoring year (May 2020). The other sites showed similar values between campaigns. Site 2F was characterized by the highest presence of PCBs and PAHs, while PAHs were mostly undetected in the other sites, except for 1F in the first year. The detected concentrations of PAHs in 1F and 2F are in line with values previously reported in the literature for the southern part of the lagoon (Apitz et al., 2007; Cassin et al., 2018; Moschino et al., 2012). The concentration of PAHs can be a fingerprint of fossil fuel combustion that in the work of Cassin et al. (2018) was associated to the fishing boat traffic characterizing the nearby city of Chioggia. Site 1F and 2F are also located in proximity to the lagoon inlet with a likely more intense transit of boats in comparison to the two innermost sites.

The application of the chemical LoE well summarized the observed trends. The hazard resulted severe for 1F, moderate for 2F, slight for both 3F and 4F in May 2020. The hazard in May 2021 was classified as absent for 1F, moderate for 2F and slight for both 3F and 4F. Table 1 summarizes the application of the methodology to the chemical data, providing additional information on the number of parameters exceeding the threshold and their contribution to the cumulative hazard class for the two selected times.

3.2. Bioaccumulation in Manila clams

Concentrations of trace elements measured in clams’ soft tissues did not present noteworthy differences among sites, with values of the same order of magnitude as those measured at seeding time (Supplementary material SM2). Concentrations were higher in the second year, with the highest differences shown by Cd, Ni, and Pb, and the lowest ones by As. Conversely, Cr concentrations in clams were comparable in the two years and were more than one order of magnitude lower than in sediments, confirming the very low bioavailability of this metal.

Concentrations of PCDD/Fs and PAHs in clams showed in general higher values in May 2021 compared to May 2020, but the observed differences could be affected by the very low number of detected congeners for these pollutants. The difference between the two years was instead less marked for PCBs, for which the levels remained similar, or slightly higher in 3F, to those initially present in seed. Organisms from site 3F generally reported higher concentrations of various contaminants, which at first sight did not seem to relate to concentrations measured in sediments. Sediments from the ambient site were indeed the least contaminated ones. This result is not new in literature. It has been observed in previous studies that bivalves inhabiting polluted areas of the Venice lagoon showed concentration in soft tissues not consistent with chemical concentrations detected in sediments and in the water column (Ademollo et al., 2017; Moschino et al., 2012).

When compared to the organisms reared in ambient conditions, the hazard was classified as slight for all farming sites in May 2020. In the second year, hazard was absent for 1F and 4F and slight for 2F. Among sites, 2F presented the greatest hazard scores that in the first May was a result of the concentration of dioxins together with dioxin-like PCBs (Table 1). However, the concentrations are well below the EQS for biota as defined in the Italian Decree 172/2015, which complies with the Directive 2013/39/EU (EU 2013). Compliance with the EQS values for human consumption of fishery products confirms that the obtained hazard classification is representative of the limited bioavailability of chemicals in the farming areas.

3.3. Biomarker analysis results

Considering the gills and digestive track, all biomarkers presented...
Table 1

HIs and classes of hazard for the four LoEs for each site/time. In chemistry LoE, information about the number of exceeding parameters and contaminants majorly contributing to the HI is reported. AMB = ambient condition, C = parameter's average concentration, EQS = sediment environmental quality standard, w = parameters weight. In biomarker LoE, ’g’ and ’dg’ stand for gills and digestive gland, respectively.

<table>
<thead>
<tr>
<th>LoE</th>
<th>1st year, May 2020</th>
<th>2nd year, May 2021</th>
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<tbody>
<tr>
<td></td>
<td>3F-AMB</td>
<td>1F</td>
</tr>
<tr>
<td></td>
<td>SLIGHT</td>
<td>SEVERE</td>
</tr>
<tr>
<td></td>
<td>Exceeding parameters</td>
<td>1</td>
</tr>
<tr>
<td>Chemical with (C · w/EQS)_{max}</td>
<td>Cr</td>
<td>Benzo(b)fluoranthene</td>
</tr>
<tr>
<td>% (C · w/EQS)_{max}/HI</td>
<td>1.45</td>
<td>3.28</td>
</tr>
<tr>
<td>Class</td>
<td>SLIGHT</td>
<td>SLIGHT</td>
</tr>
<tr>
<td>HIs</td>
<td>150.0</td>
<td>191.7</td>
</tr>
<tr>
<td>Moderate parameters</td>
<td>-</td>
<td>PCDD/Fs + DL PCBs</td>
</tr>
<tr>
<td>Major parameters</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Severe parameters</td>
<td>-</td>
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<th>LoE</th>
<th>1st year, May 2020</th>
<th>2nd year, May 2021</th>
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<tbody>
<tr>
<td></td>
<td>3F-AMB</td>
<td>1F</td>
</tr>
<tr>
<td></td>
<td>SLIGHT</td>
<td>MODERATE</td>
</tr>
<tr>
<td></td>
<td>86.25</td>
<td>161.25</td>
</tr>
<tr>
<td>Moderate parameters</td>
<td>SOD g</td>
<td>-</td>
</tr>
<tr>
<td>Major parameters</td>
<td>-</td>
<td>-</td>
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<td>Severe parameters</td>
<td>-</td>
<td>SOD g</td>
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<th>LoE</th>
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<tr>
<td></td>
<td>MODERATE</td>
<td>MODERATE</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>88.8</td>
</tr>
<tr>
<td>Major categories</td>
<td>Development</td>
<td>Proliferation Metabolism</td>
</tr>
<tr>
<td>Severe categories</td>
<td>-</td>
<td>-</td>
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significant differences between the two years, except for GST (Supplementary material SM1-Table S3). SOD values in 1F and 2F, closer to the inlet, were significantly higher in May 2020 compared to May 2021. In the first year, the activation of SOD was particularly pronounced for 2F suggesting a response of organisms to an oxidative stress. In the second year, CAT analysed in gill presented significant differences in all sites with greater inhibition in 4F compared to animals from 3F. GR activity, which was significantly lower in the second year in both gill and digestive gland (Fig. S2-E, F), presented significant variations in sites 1F and 2F when compared to the ambient site. AChE activity values were significantly lower in the second year and in particular in site 2F which showed the lowest values (Fig. S2-G). Site 2F had also significantly higher GST activity values compared to the ambient site 3F.

The results of biomarkers hazard assessment are summarized by the HIs presented in Table 1 along with additional information of parameters identified by moderate, major, or severe class of effect. Sites 1F and 4F presented slight hazard in May 2020, whereas hazard for site 2F was classified as moderate. Due to changes in GR and AChE activities mainly, a general worsening characterized the second year, where 1F and, particularly, 2F reported major hazard. 4F hazard increased too, being it moderate in May 2021.

The worsening of the condition from the first to the second year reflects the increase of concentration in biota observed in the previous line. The higher hazard might also depend on other compounds that were not measured in sediment and clams’ soft tissues. The changes in AChE (which plays a key role in the transmission of nerve signals) detected in the second year suggested the presence of neurotoxic compounds which can inhibit its activities (Guo et al., 2021; Matozzo et al., 2005; Rilievo et al., 2021). The other biomarkers analysed could be triggered by many forms of contamination in bivalves, from nanoparticles to pharmaceuticals (Marisa et al., 2022; Matozzo et al., 2012), as well as seawater parameters (Munari et al., 2018; Pokhrel et al., 2021). Nonetheless, for both analysed times biomarker analyses suggest that 2F was the most affected site with clams showing the greatest changes in SOD, CAT, GR, GST, and AChE.

3.4. Transcriptomics analysis results

The most important transcriptional changes in Manila clam were observed in farming sites placed close to Chioggia inlet (1F and 2F) in
the first monitoring year, resulting in moderate hazard in May 2020. This result confirmed the most important findings highlighted by Milan et al. (2023). In that study, the disruption of clam's inflammatory immune response, cell cycle, and apoptosis regulation, and the up-regulation of genes and molecular pathways involved in xenobiotic metabolism were observed in clams farmed in sites close to the Chioggia inlet (i.e. 1F and 2F). The alterations suggest major environmental stressors affecting these sites, where the highest mortality was also reported by Milan et al. (2023). Here, the application of HALLMARK gene sets revealed in 1F and 2F the disruption of key signalling pathways (i.e. mTORC1 signalling) and cell cycle regulation (i.e. MYC targets; G2M checkpoint), an increase in inflammatory response, xenobiotic metabolism and energy metabolism (i.e. oxidative phosphorylation and fatty acid metabolism) at the expenses of pathways associated to development, proliferation and metabolism.

Clams reared in 4F presented slight transcriptional hazard, which was maintained also in the second year. On the contrary, modifications at transcriptional levels were less pronounced in May 2021 for clams from sites 1F and 2F when compared to the first year. Despite the fact that 1F and 2F were characterized by slight hazard, greater transcriptional changes were observed in clams from 2F, in which moderate effect was assigned to pathways related to development (Table 1).

3.5. Integration of LoEs

Chemistry, bioaccumulation, biomarkers, and transcriptomics LoEs contributed to the assessment of three of the most important farming sites (1F, 2F, 4F) in the Venice lagoon, whereas for site 3F that acted as ambient site for bioaccumulation, biomarker, and transcriptomics analyses, the evaluation of its good quality status was mostly supported by the chemistry evidence. Table 2 reports the conclusive HIWoE in May 2020 and 2021, along with the overall class of hazard for all investigated sites. In the first year (May 2020), sites close to the Chioggia inlet (i.e. 1F and 2F) scored higher hazards (i.e. moderate) than 3F and the site furthest away from the inlet, 4F, both scoring slight. After a year, all sites presented slight hazards, with the exception of site 2F which saw the first-year moderate level of hazard confirmed.

For visual inspection, LoE scores are plotted in circular diagrams (Fig. 2), where the sector width indicates the weight of each LoE with respect to the overall HIWoE and the radial extension denotes the LoE magnitude of hazard converted into a 0–100 scale. The plots allow to identify the extent of agreement or dissimilarity across different bodies of evidence as discussed in the next section.

3.6. Integrating WoE results with environmental parameters

In all sites, with the exception of 1F whose quality improved, no remarkable differences emerged between the two campaigns if the overall hazard quotients and classes are considered.

Site 2F resulted as the most impacted farming area reporting moderate hazards in both year. In this site, sediment quality determined the final hazard with the contribution of transcriptomics in May 2020 and biomarkers in May 2021. Sediment chemical characterization can provide key information to define the quality of farmed clams and explain possible causes of stress, but it is not the only factor playing a role in defining the quality of the sites. In environments where contamination is not particularly high, transcriptional and biomarker profiles can be influenced by several biotic and abiotic factors. It is then important to consider other parameters in the assessment in order to attain a proper interpretation of the results (Cravo et al., 2012).

Looking at the data from the two multiparametric probes (Fig. 3), the daily and annual average of water temperature and salinity remained practically invariant across the two years (Table S7 in SM1). However, differences emerged between the two probes, that is, between sites. By
visualizing probes measurements with values defined as optimum range for this species (Paesanti and Pellizzato, 2000), probe North close to site 1F and 2F reported a higher frequency of exceedance of the optimum range for salinity and temperature. Salinity measured by Probe North was recurrently greater than 35 ‰, which corresponds to the highest value associated to clams’ optimum status. Clams farmed closer to the lagoon inlet not only experienced a higher level of thermal and salinity stress, but were also exposed to values above the optimum for a more prolonged time than clams in the innermost sites, particularly during the first monitoring year.

Higher salinity and water temperature may also favor the proliferation of pathogens (La Peyre et al., 2006; Paillard et al., 2004), which is one of the causes behind the increase in mortality observed in farming areas within the Venice Lagoon (IZS VE 15/13, 2017) and in other parts of the world (Smits et al., 2020; Nam et al., 2018). Such differences may have repercussions on the health status of clams, thus explaining the wider variability and higher hazard levels for sites closer to the inlet (i.e. 1F and 2F) than for sites located further away in the lagoon (i.e. 4F). This information should assist in defining aquaculture future development and management plans.

It is also important to note that COVID19 global pandemic occurred during the two investigated years. On top of this, further complexity is added to the whole picture by the meteorological and environmental conditions in general, the activation of the MoSE storm-surge barriers, and the hydraulic and mechanical dredges used in bivalve farming (Losso and Ghirardini, 2010). Alone or in synergy, these perturbation factors might have influenced the sediment transport processes and quality, and the water physicochemical characteristics. However, temperature, salinity, and contaminants concentration in these two years fluctuated within the range of variability reported in previous studies and no significant differences emerged for contaminants measured in sediments in May 2020 (during the lockdown), as showed in Fig. S1 in SM1. Climate change impacts and MoSE effects on the lagoon morphology and physicochemical characteristics might be visible on the long term (Tognin et al., 2022), so this preliminary study can set the baseline for future monitoring plans.

### 3.7. Application of an integrated approach

Moving beyond this case study, the WoE approach proved to be an effective tool that can be employed also in the assessment of farming areas in general. Several studies have advocated for the application of integrated approaches to better interpret signals of clam stresses (Cravo et al., 2012; Soon and Ransangan, 2019). WoE approaches are time-consuming and resource-intensive, but they offer to picture the quality status of a site in a more complete way. If the assessment of the farming areas had relied on sediment chemical analysis alone, the response of organisms to sediment contamination and others site-specific environmental stressors would have been hindered. Likewise, basing the assessment on a single biological index, such as the Condition Index or the biomarker response, might have led to a misinterpretations of data. A single index is the result of a complex balance between endogenous and exogenous factors (Sasikumar and Krishnakumar, 2011), thus masking the main drivers of stress. On the contrary, in a QWoE approach moving backward, from the final class of hazard to the original data that contributes to each LoE, is possible and it can assist in linking the assessment to the stressors impacting on the bivalve farming areas.

The assembly of different bricks of evidence would have been quite challenging without the use of a structured Weight of Evidence (WoE) framework. The conceptual framework and algorithms used here can be easily applied to other aquaculture areas, with the only requirements of adapting the sediment EQSs to the appropriate local or national guidelines and selecting the correct ambient site. Bioaccumulation, biomarkers, and transcriptomics evidence is gathered by comparative analysis, i.e., comparing a sample with a control. To acknowledge the difficulty of identifying a control sample in field applications, it was here preferred the term “ambient” condition for site 3F (Rüllig et al., 2022). When considering the final results, one should then keep in mind that the risk assessment resulting from the different types of biological evidence is derived by comparison with the “ambient” condition of a specific site exploited for Manila clam farming.

A WoE approach occurs to be successful also in the inclusion of new types of evidence with the intent of reaching a more robust assessment. For the first time in situ assessments, transcriptional alterations were
quantitatively elaborated to take part in the inference process. Transcriptomics constituted a further independent line and proved the possibility of introducing genomic-based evidence in structured assessments, something that has been encouraged for long time (Soufan et al., 2022). In this study, the quality of farming sites was preliminary evaluated with a WoE approach that considers contamination as one of the main drivers of stress. It is believed that this constitutes a first layer of information regarding the overall quality of farming sites that has been often overlooked. However, it is not exhaustive. The biological evidence gathered in this study pointed also to other factors potentially affecting the physiological status of animals, such as emerging contaminants not yet regulated, physicochemical properties of the farming environments, and the potential presence of pathogens. A more thorough assessment calls for the future development of other indexes embodying the influence of environmental and biotic parameters to be integrated in a broad WoE approach for the assessment of the quality of shellfish farming sites.

Fig. 3. Daily averaged values of temperature (°C) and salinity (‰) measured by (A) probe North and (B) probe South. Optimum and vital ranges for both parameters are presented. Light blue vertical stripes are in correspondence of MoSE operation occurrence. Brown shaded area indicates the cumulative stress, defined as the amount of time spent in suboptimal conditions (i.e. when the considered parameter exceeds the optimum range). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
4. Conclusions

A QWoE approach was proposed to assess the quality of bivalve farming sites. The experimental investigation comprised four sites of clam farming activities at different distances from one of the Venice lagoon inlets allowing the construction of four independent LoEs (i.e., chemistry, bioaccumulation, biomarker, and transcriptomics) to account for sediment chemistry-based evidence but also adverse effects at the organism level.

After evidence aggregation, differences emerged when location of the farming sites was accounted for, with sites closer to the open sea presenting important modifications at transcriptional and biomarker levels. The physicochemical evidence collected and the presence of several factors perturbing the farming areas, advise against inferring that the sediment quality alone was responsible for the observed responses. To pinpoint other driving factors, additional indexes indicative of biotic and abiotic data should be included in future integrated frameworks.

The QWoE approach presented here explored this possibility by integrating the sediment quality and the response of farmed clams at different biological levels, such as that described by new line of evidence based on transcriptional alterations. However, this is a preliminary evaluation of the integrated approach to aquaculture that should lead the way to a more exhaustive spectrum of evidence. With the objective of improving the robustness of the assessment of farming sites quality, this is something to consider in a future scenario of climatic changes and frameworks.

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