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From a validated targeted method to a retrospective UHPLC-HRMS non-targeted analysis unveiling COVID-19-related contaminants in clams. Have we bias in marine model organisms for ecotoxicological studies?

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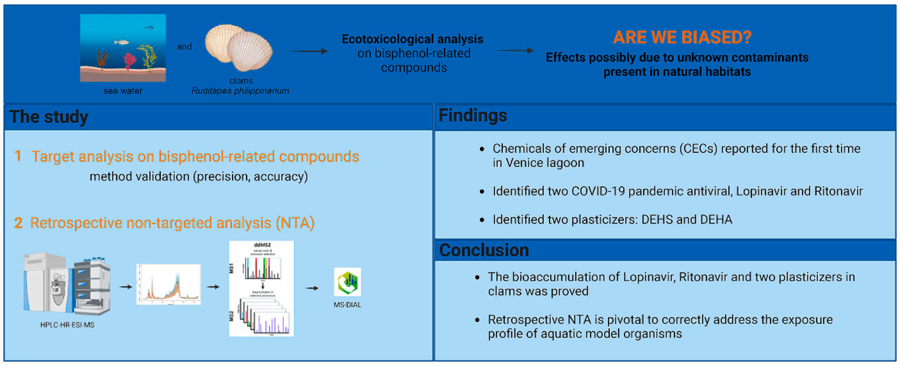
HIGHLIGHTS

- Retrospective analysis of seawater and clams by NTA HMRS shows unexpected emerging contaminants.
- Reliable NTA requires good accuracy and reproducibility on targeted analytes for quality assurance.
- Lopinavir, Ritonavir and two plasticizers reported for the first time in the Mediterranean Sea.
- The bioaccumulation of Lopinavir, Ritonavir and two plasticizers in clams was proved.
- Retrospective NTA is pivotal to correctly address the exposure profile of aquatic model organisms.

GRAPHICAL ABSTRACT



Findings

- Chemicals of emerging concerns (CECs) reported for the first time in Venice lagoon
- Identified two COVID-19 pandemic antiviral, Lopinavir and Ritonavir
- Identified two plasticizers: DEHS and DEHA

Conclusion

- The bioaccumulation of Lopinavir, Ritonavir and two plasticizers in clams was proved
- Retrospective NTA is pivotal to correctly address the exposure profile of aquatic model organisms

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ABSTRACT

This study explores a retrospective non-targeted analysis (NTA), based on Ultra High-Performance Liquid Chromatography coupled to High-Resolution Mass Spectrometry (UHPLC-HRMS), to assess hidden chemicals of emerging concern (CECs) in marine model organisms. Conventional ecotoxicological studies do not include evaluating the natural habitats of the collected organisms, missing the possibility of highlighting unexpected pollutants, and thus compromising the correctness and reliability of the experimental results. In this paper we reprocessed samples previously collected from the Venice Lagoon for ecotoxicological studies and used for targeted analysis of three bisphenols-related compounds (i.e. BPS, BPF and BPAF) on seawater and specimens of the clam *Ruditapes philippinarum*. Results from the validation were the following: accuracy, expressed as percentage recoveries (R%), in the range 80%<R%<120% for all the considered compounds and matrices, and precision, expressed as relative standard deviation of the absolute areas of IS, was <20% for clams (N = 24) and <15% for seawater (N = 30). LODs ranged from 5 to 50 ng/L for seawater and 5–12 ng/g for clam tissues.

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After validation, a retrospective NTA was carried out on control samples, showing the presence of some CECs, i.e. Lopinavir, Ritonavir, DEHS, and DEHA, two antiviral drugs used during the COVID-19 pandemic and two plasticizers, respectively, reported here for the first time in the Mediterranean Sea. CEC identifications were confirmed by matching MS/MS spectra with libraries. The present study emphasizes the importance of retrospective investigations to describe the contamination scenario of environmental matrices and the related effect in biota, to correctly address studies on model animals, also including possible “cocktail effects”.

1. Introduction

In ecotoxicological studies, marine organisms are commonly used in both laboratory and field conditions to assess both the presence and the effects of contaminants. In particular, filter-feeder molluscs are suitable models for understanding the effects of contaminants on their physiology and metabolism (Impellitteri et al., 2022; Świacka et al., 2019). While a good ecotoxicological model should answer to technical criteria such as easy laboratory maintenance, fast growth, and the availability of different experimental assays (Segner and Baumann, 2016), the animal source is often not considered. Except for those animals grown in a laboratory under controlled conditions, the reference organisms used as controls and for exposure studies are normally collected in “polluted-free” areas. However, such sampling areas are not subjected to a routinary analytical monitoring to control possible pollution events, and collected model organisms might not be free of contaminants, resulting in a false natural environment baseline. If this lack of analytical surveys is paired with the possible presence of chemicals of emerging concern (CECs), the answer to the ecotoxicological experiments might be impaired.

Considering these perspectives, High-Resolution Mass Spectrometry (HRMS) is a sensible investigation tool to identify possible contaminants in environmental matrices and animal tissues (Badea et al., 2020; Menger et al., 2020). Already collected and processed ecotoxicological samples are in principle suitable to perform a retrospective analysis to highlight the presence of suspect and unknown contaminants; anyway, the sample preparation procedures must be properly validated, since some quality assurance (QA) measures are necessary to empower further sample comparison and the statistical evaluation. In this paper we re-processed samples previously collected and analysed by a validated method for targeted analysis of known contaminants, to obtain information also on other pollutants by a retrospective non-targeted analysis (NTA). The target analysis was applied to seawater samples and specimens of the clam *Ruditapes philippinarum* collected from the Venice Lagoon basin for ecotoxicological studies on the effects of bisphenol analogues (Fabrello et al., 2023; Fabrello and Matozzo, 2022). After validation, control clams were re-analysed by an Ultra High Performance Liquid Chromatography (UHPLC)-HRMS based non-targeted approach.

2. Experimental section

2.1. Samples collection

Seawater samples were collected in the southern basin of the Lagoon of Venice (Italy), near Chioggia (45°13′ latitude N, 12°17′ longitude E), in summer 2020 (Fig. S1). Adults of *R. philippinarum* (4.5–5 cm shell length) were collected in the Venice Lagoon (surroundings of 45°14′ latitude N, 12°16′ longitude E). Maintenance conditions are reported elsewhere (Rilievo et al., 2021). Soft-body tissues of samples used as negative controls for previous ecotoxicological studies (Fabrello et al., 2023) from three pools of 5 clams each, were excised, frozen in liquid nitrogen and stored at −20 °C. In addition, three 100 mL seawater samples were collected from the aquaria used for the exposure phase and similarly stored. The collected samples were used for the preliminary method development aimed to study ecotoxicological effects on bisphenols-related compounds, namely BPS, BPF and BPAF (Fabrello et al., 2023). For both water and tissue samples treatment, see the Supplementary data.

2.2. Bisphenols method validation in water and tissue samples

The accuracy of the method for seawater samples, intended as a combined recovery and matrix effect, was assessed evaluating the percentage ratio between the slope of a seven-points matrix-matched calibration curves built by spiking 100 mL of artificial seawater (ASTM Standards D 1141–98) with standards (BPS, BPF and BPAF in the range 20–600 ng/L) and internal standard (IS, bisphenol A d-16, 300 ng/L) before the extraction procedure, and the slope of a seven-points calibration curve built by diluting standards and IS in methanol at the corresponding concentrations. Blank samples, made of artificial seawater spiked with IS at the final concentration of 300 ng/L, were extracted and analysed to determine possible system contaminations for QA purpose.

For animal tissues the same validation approach for seawater samples was followed. Combined recovery and matrix effect were evaluated by comparing the slope of a six-points matrix-matched calibration curve, obtained spiking control clam tissues (blank samples) before the homogenization (BPS, BPF and BPAF in the range 5–100 ng/g; IS at 50 ng/g), with that of a six-points calibration curve built by spiking standards and IS in methanol at the corresponding concentrations. All calibration curves were analysed in triplicate and plotted using analyte peak areas normalized by the IS ones.

2.3. UHPLC-HRMS

UHPLC analysis was performed by an Ultimate 3000 (Thermo Fisher Scientific, Germany) system, with a Kinetex Polar EVO C18 (100 mm × 2.1 mm, 2.6 μm, 100 Å; Phenomenex, CA, USA) column at 30 °C temperature. The elution solutions were 0.1% formic acid in deionized water for the aqueous phase (A), and 0.1% formic acid in acetonitrile for the organic phase (B). The elution gradient was: 0–3 min, 2% B; 3–18min, 2% B–100% B; 18–20 min, 100% B; 20–21 min, 100% B–2% B; 21–30 min, 2% B. A flow of 0.250 mL/min was used, and the injection volume was 10 μL.

Data acquisition was performed by Q-Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, Germany) full scan-ddMS2, in both positive and negative ionization. For positive ionization, the capillary voltage was set to 4.0 kV, capillary temperature was 320 °C, auxiliary gas and sheath gas were nitrogen (300 °C) at 20 and 10 psi, respectively. Data acquisition was performed in profile mode. Resolution was set to 70000 in MS mode and 35000 in ddMS2 mode, while AGC target was 1×10^6 in MS mode and 1×10^5 in ddMS2 mode, max injection time 250 ms in both scan modes. Full-scan range was within *m/z* 150–2000. ddMS2 experiments were performed by selecting the 5 most abundant species in the previous MS spectrum with a minimum AGC target was 1×10^4, and by setting an isolation window of 2 Da and a normalized collision energy of 35. For negative ionization, parameters were kept the same as positive ionization, except for capillary voltage that was 2.8 kV. Calibration was performed with Pierce ESI Positive Ion Calibration Solution (Thermo Fisher Scientific, Germany) and Pierce ESI Negative Ion Calibration Solution (Thermo Fisher Scientific, Germany).

Table 1

Method validation parameters calculated for BPF, BPAF and BPS, using artificial seawater as matrix for seawater samples and blank clam homogenate as matrix for tissue samples. Three replicates were prepared for each of the seven (seawater samples) and six (clam samples)-point matrix calibration curves, in the range of 20–600 ng/L for water sample (IS at 300 ng/L), and 5–100 ng/g (IS at 50 ng/g) for clam tissue.

	Slope (relative standard deviation) of the matrix-matched calibration curve			Accuracy, R%			LOD [ng/L] or ng/g		
Analytes	BPF	BPAF	BPS	BPF	BPAF	BPS	BPF	BPAF	BPS
Seawater	0.00459 (8.5%)	0.000324 (3.4%)	0.000711 (8.7%)	97%	118%	107%	50	5.0	20
Clam tissues	0.00154 (2.3%)	0.03891 (2.0%)	0.00621 (2.6%)	81%	90%	90%	12	5.2	8.0

2.4. Targeted and retrospective non-target analysis

Raw data were converted in.abf format by the ABF Converter software and files were further processed by MS-DIAL (version 4.9.221218). Used parameters for peak detection, in both positive and negative ESI ionization mode, were MS1 and MS2 tolerance of 0.005 Da and minimum peak height of 50000. Considered adducts were $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$ and $[M+NH_4]^+$ in positive mode, and $[M-H]^-$ and $[M+COOH–H]^-$ in negative mode. Alignment parameters were retention time tolerance 0.8 min and MS1 tolerance 0.005 Da. Databases selected for feature identification were MSMS_Public_EXP_Pos_VS17 (reporting 16481 unique compounds) in positive ionization mode and MSMS_Public_EXP_Neg_VS17 (reporting 9033 unique compounds) in negative ionization mode; identification parameters were accurate mass tolerance (MS1) 0.005 Da, accurate mass tolerance (MS2) 0.01 Da and identification score cut off 80%. Annotated compounds (identification score <80%) were excluded for further evaluation, as well as identified or annotated compounds present also in system blanks. Identified compounds (presence of both MS and MS2 spectra, and identification score >80%) were manually validated to ensure the trueness of the matching between the experimental MS2 spectrum of the considered features and the one reported in databases, as well as correct integration of peaks. The identification of these features was further confirmed by comparing experimental MS2 spectra with the mzCloud database. BPS, PBF, BPAF and IS peak areas were manually integrated from $[M-H]^-$ extracted ion chromatograms (mass accuracy 5 ppm).

3. Results and discussion

Validation parameters were calculated for BPS, BPAF and BPF, as they were the analytes considered in the initial targeted analysis. Results for validation for seawater and clams' homogenates are reported in Table 1. Linearity, reported as the regression coefficient R^2 for a linear least square fitting, was excellent for each analyte in both cases (R^2 higher than 0.997). Good to excellent accuracies were also obtained, indicating overall good percentage recoveries and absence of matrix effect for all the target compounds. Precisions of methods were estimated by the relative standard deviation (RSD) of the IS absolute peak areas in all the analysed samples; RDS was lower than 15% for seawater ($N = 30$) and lower than 20% for clam tissues ($N = 24$). LODs, assessed as $S/N = 3$ from the lowest concentration of the calibration curves, ranged from 5 to 50 ng/L for seawater and from 5 to 12 ng/g for clam tissues, thus indicating a quite variable ESI molar response factor. In this light, a quite large variation of LOD responses must be considered also for unknown compound, depending on their chemical characteristics (i.e. molecular weight and polarity).

Considering the further retrospective NTA, the validation of such a method by considering recovery, matrix effect and LOD for targeted compounds always lead to an incomplete evaluation, as it is not possible to extrapolate accuracy and sensitivity for unknown analytes. In principle, it could be possible to extent the validation process to a larger panel of molecules, considering species characterized by a wider range of polarities and molecular weights. Anyway, results will be still strictly related to the molecules considered, making the workflow more expensive and time-demanding. On the other hand, ensuring the reproducibility and robustness of the extraction procedures is pivotal to allow the statistical comparison of different samples extracted and processed with the same protocol. Results obtained in this study were limited to a single IS (RSD <20% on absolute areas), but still very satisfying also from an NTA and retrospective point of view. In this framework, it will be desirable to extend the evaluation of precision and robustness of the general analytical method by considering as many IS as possible. In particular, IS should be chosen in order to cover the full-time duration of the chromatographic separation (i.e. ISs eluting at different retention times) and both ESI acquisition polarities (Hu et al., 2016).

After the targeted analysis, acquired data for extracted seawater were then processed with MS-DIAL for NTA, matching the experimental information with libraries containing more than 25000 compounds. The negative acquisition did not yield any identified compound. Differently, the positive acquisition highlighted some anthropogenic compounds which were not yet reported in the Venice lagoon. Firstly, two antivirals, namely Lopinavir and Ritonavir, were detected and annotated by mass spectrum matching with reported libraries. Together with Favipiravir, Remdesivir and hydroxychloroquine, they are some of the drugs used during the COVID-19 emergency (Gavriatopoulou et al., 2021; McKee et al., 2020), and their concentrations in environmental waters were predicted in the order of hundreds to thousands ng/L (Czech et al., 2022; Kuroda et al., 2021), also because they are recalcitrant to wastewater treatments (Efrain Merma Chacca et al., 2022; Majumder et al., 2021; Morales-Paredes et al., 2022). These two antivirals were already detected in surface waters of COVID-19 high-affected areas (Zhang et al., 2022).

In addition, two synthetic diesters were confirmed, i.e. di-(2-ethylhexyl)-sebacate (DEHS) and di-(2-ethylhexyl)-adipate (DEHA). They are used as plasticizer to increase the technical and durability properties of polymers (Fankhauser-Noti and Grob, 2006; Hahladakis et al., 2018). MS2 spectra of the four contaminants are reported in the Supplementary data (Figs. S2–S5).

Due to the presence of such contaminants in the collected seawater, the control clams cultured in aquaria were re-processed to verify the potential accumulation of this species in animal tissues. The presence of the four water contaminants (*i.e.*, Lopinavir, Ritonavir, DEHS and DEHA) was confirmed also in the tissue samples of the exposed clams, supporting the hypothesis of a bioaccumulation from the lagoon seawater during the exposure in tanks. Other features were identified, for the most part they were fatty acids, naturally present in clam tissues, or biochemical metabolites.

It is worthy to notice that seawater samples for the exposure experiment were collected in summer 2020, and control clams were frozen and successively used for the bisphenols study. The use of Lopinavir and Ritonavir during the pandemic crisis combined with a possible inefficiency of the wastewater treatment plant (WTP) located in Chioggia, may have contributed to their release into the lagoon waters. In this context, it is important to highlight that WTPs are not always able to remove all chemicals, such as pharmaceuticals, from wastewater (Bijlsma et al., 2021).This is the first report on the presence of Lopinavir and Ritonavir used during COVID-19 pandemic in the Mediterranean seawater.

4. Conclusions

In this short communication, we provided hints on how a retrospective NTA can be useful in reprocessing samples for uncovering hidden contaminants, providing valuable insights into the complexities of environmental exposures. The unexpected presence of several CECs, such the antiviral pharmaceuticals and plasticizers, i.e. Lopinavir, Ritonavir DEHS, and DEHA, reported for the first time into the Venice lagoon, emphasizes the importance of incorporating retrospective investigations to reconsider common ecotoxicological well-established aspects and protocols. As a matter of fact, all the ecotoxicological evaluations about effects related to the exposure to certain contaminants are comparative with respect to control samples, which are indeed considered as pollution-free. This insight into the presence of unconsidered CECs also empower the need of the evaluation of possible synergic effects. In this light, it will be necessary to review the general approach applied in ecotoxicological evaluations until now. In particular, when model animals and maintenance/exposure media (e.g., seawater) are taken from the environment, a proper NTA should be considered, in order to avoid inaccuracies of the final results. As we delve into the intricate world of CECs, HRMS non-targeted analyses continue to be an invaluable tool for expanding our knowledge and addressing critical questions in ecotoxicology.

Anyway, some aspects remain critical for NTA validation, and are here summarised:

- Extraction procedures drive the class, the number and LODs of the molecules that can be successively identified;
- Accuracy and reproducibility of the selected IS are the only parameters that can be used as QA for NTA, starting from a target approach;
- The choice of IS is recommended to be the largest possible, covering both ESI polarities and different chemical moieties and properties, but it is anyway very expensive;
- Strict quantitative analysis is not achievable, but a range of putative concentration or LODs may be proposed probably within two orders of magnitude of uncertainty, once that a larger statistical evaluation will be performed.

CRediT authorship contribution statement

Graziano Rilievo: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Samuele Boscolo:** Investigation, Formal analysis. **Silvia Pettenuzzo:** Investigation, Formal analysis. **Valerio Matozzo:** Writing – original draft, Supervision, Resources, Funding acquisition. **Jacopo Fabrello:** Investigation, Formal analysis. **Marco Roverso:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Sara Bogialli:** Writing – review & editing, Supervision, Resources, Project administration, 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.chemosphere.2024.142994.

References

Badea, S.-L., Geana, E.-I., Niculescu, V.-C., Ionete, R.-E., 2020. Recent progresses in analytical GC and LC mass spectrometric based-methods for the detection of emerging chlorinated and brominated contaminants and their transformation products in aquatic environment. Sci. Total Environ. 722, 137914 https://doi.org/10.1016/j.scitotenv.2020.137914.

Bijlsma, L., Pitarch, E., Fonseca, E., Ibáñez, M., Botero, A.M., Claros, J., Pastor, L., Hernández, F., 2021. Investigation of pharmaceuticals in a conventional wastewater treatment plant: removal efficiency, seasonal variation and impact of a nearby hospital. J. Environ. Chem. Eng. 9, 105548 https://doi.org/10.1016/j.jece.2021.105548.

Czech, B., Krzyszczak, A., Boguszewska-Czubara, A., Opielak, G., Jośko, I., Hojamberdiev, M., 2022. Revealing the toxicity of lopinavir- and ritonavir-containing water and wastewater treated by photo-induced processes to Danio rerio and Allivibrio fischeri. Sci. Total Environ. 824, 153967 https://doi.org/10.1016/j.scitotenv.2022.153967.

Efrain Merma Chacca, D., Maldonado, I., Vilca, F.Z., 2022. Environmental and ecotoxicological effects of drugs used for the treatment of COVID 19. Front. Environ. Sci.

Fabrello, J., Ciscato, M., Munari, M., Vecchiatti, A., Roverso, M., Bogialli, S., Matozzo, V., 2023. Ecotoxicological effects and bioaccumulation of BPA analogues and their mixture in the clam Ruditapes philippinarum. Mar. Environ. Res. 192, 106228 https://doi.org/10.1016/j.marenvres.2023.106228.

Fabrello, J., Matozzo, V., 2022. Bisphenol analogs in aquatic environments and their effects on marine species—a review. J. Mar. Sci. Eng. https://doi.org/10.3390/jmse10091271.

Fankhauser-Noti, A., Grob, K., 2006. Migration of plasticizers from PVC gaskets of lids for glass jars into oily foods: amount of gasket material in food contact, proportion of plasticizer migrating into food and compliance testing by simulation. Trends Food Sci. Technol. 17, 105–112. https://doi.org/10.1016/j.tifs.2005.10.013.

Gavriatopoulou, M., Ntanasis-Stathopoulos, I., Korompoki, E., Fotiou, D., Migkou, M., Tzanninis, I.-G., Psaltopoulou, T., Kastritis, E., Terpos, E., Dimopoulos, M.A., 2021. Emerging treatment strategies for COVID-19 infection. Clin. Exp. Med. 21, 167–179. https://doi.org/10.1007/s10238-020-00671-y.

Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling. J. Hazard Mater. 344, 179–199. https://doi.org/10.1016/j.jhazmat.2017.10.014.

Hu, Y.-L., Chen, Z.-P., Chen, Y., Shi, C.-X., Yu, R.-Q., 2016. Generalized multiple internal standard method for quantitative liquid chromatography mass spectrometry. J. Chromatogr. A 1445, 112–117. https://doi.org/10.1016/j.chroma.2016.04.008.

Impellitteri, F., Curpăn, A.-S., Plăvan, G., Ciobica, A., Faggio, C., 2022. Hemocytes: a useful tool for assessing the toxicity of microplastics, heavy metals, and pesticides on aquatic invertebrates. Int. J. Environ. Res. Publ. Health. https://doi.org/10.3390/ijerph192416830.

Kuroda, K., Li, C., Dhangar, K., Kumar, M., 2021. Predicted occurrence, ecotoxicological risk and environmentally acquired resistance of antiviral drugs associated with COVID-19 in environmental waters. Sci. Total Environ. 776, 145740 https://doi.org/10.1016/j.scitotenv.2021.145740.

Majumder, A., Gupta, A.K., Ghosal, P.S., Varma, M., 2021. A review on hospital wastewater treatment: a special emphasis on occurrence and removal of pharmaceutically active compounds, resistant microorganisms, and SARS-CoV-2. J. Environ. Chem. Eng. 9, 104812 https://doi.org/10.1016/j.jece.2020.104812.

McKee, D.L., Sternberg, A., Stange, U., Laufer, S., Naujokat, C., 2020. Candidate drugs against SARS-CoV-2 and COVID-19. Pharmacol. Res. 157, 104859 https://doi.org/10.1016/j.phrs.2020.104859.

Menger, F., Gago-Ferrero, P., Wiberg, K., Ahrens, L., 2020. Wide-scope screening of polar contaminants of concern in water: a critical review of liquid chromatography-high resolution mass spectrometry-based strategies. Trends Environ. Anal. Chem. 28, e00102 https://doi.org/10.1016/j.teac.2020.e00102.

Morales-Paredes, C.A., Rodríguez-Díaz, J.M., Boluda-Botella, N., 2022. Pharmaceutical compounds used in the COVID-19 pandemic: a review of their presence in water and treatment techniques for their elimination. Sci. Total Environ. 814, 152691 https://doi.org/10.1016/j.scitotenv.2021.152691.

Rilievo, G., Fabrello, J., Roverso, M., Bogialli, S., Matozzo, V., 2021. Effects of the fragrance galaxolide on the biomarker responses of the clam ruditapes philippinarum. J. Mar. Sci. Eng. 9 https://doi.org/10.3390/jmse9050509.

Segner, H., Baumann, L., 2016. What constitutes a model organism in ecotoxicology? Integrated Environ. Assess. Manag. 12, 199–200. https://doi.org/10.1002/ieam.1727.

Świacka, K., Maculewicz, J., Smolarz, K., Szaniawska, A., Caban, M., 2019. Mytilidae as model organisms in the marine ecotoxicology of pharmaceuticals - a review. Environ. Pollut. 254, 113082 https://doi.org/10.1016/j.envpol.2019.113082.

Zhang, Z., Zhou, Y., Han, L., Guo, X., Wu, Z., Fang, J., Hou, B., Cai, Y., Jiang, J., Yang, Z., 2022. Impacts of COVID-19 pandemic on the aquatic environment associated with disinfection byproducts and pharmaceuticals. Sci. Total Environ. 811, 151409 https://doi.org/10.1016/j.scitotenv.2021.151409.