

BIOCHEMICAL RESPONSES OF AQUATIC ORGANISMS TO EMERGING ENVIRONMENTAL CONTAMINANTS: IMPLICATIONS FOR ECOSYSTEM HEALTH AND SUSTAINABILITY

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ABSTRACT

The occurrence of emerging environmental contaminants is being observed more frequently in aquatic environments, which can cause biochemical, developmental, endocrine, physiological, and reproductive effects on aquatic species. It is necessary to know the differences between toxicant classes and biological effects types for ecological health evaluation and sustainable environmental management. This study evaluated biochemical responses of aquatic organisms to emerging environmental contaminants by comparing toxicity thresholds across contaminant categories, life stages, and biological endpoints. A quantitative secondary analysis was conducted using an ecotoxicological dataset containing contaminant exposure and biological response records for fish species. After data screening, 491 observations were retained. The lowest observed adverse effect concentration (LOAEC) was used as the primary toxicity endpoint and log₁₀-transformed before analysis. Descriptive statistics, Kruskal–Wallis tests, post-hoc pairwise comparisons, bootstrap confidence intervals, and principal component analysis were applied. Hormone-related contaminants were the most represented group, while developmental, endocrine, and physiological responses were the dominant biochemical endpoints. Toxicity thresholds differed significantly among contaminant categories, life stages, and biochemical response categories. PFAS and plasticizers showed comparatively lower median toxicity thresholds, suggesting greater biological sensitivity. Early life stages exhibited lower thresholds than adults, indicating increased vulnerability during development. Principal component analysis indicated that hormone-related and developmental response pathways contributed to major patterns of variability. The emerging chemicals have different biological impacts on aquatic species, where toxicity depends on the category of contaminant, life stage of the organism, and type of endpoint being studied. Combining toxicity levels and biological impacts would enhance aquatic risk assessment.

KEYWORDS: emerging contaminants, aquatic organisms, biochemical responses, LOAEC, ecosystem health

INTRODUCTION

Pharmaceuticals, personal care products, endocrine-disrupting chemicals, industrial chemicals, plastic additives, pesticides and per- and polyfluoroalkyl substances (PFAS) are just a few of the many emerging contaminants of concern (CECs) that are affecting aquatic ecosystems in recent years. These pollutants are continually added to fresh water and marine systems from municipal wastewater discharges, industry, agricultural runoff and urban development. Emerging contaminants can be present in the environment at low levels, but can have (chronic) biological effects, can persist in the environment, and may bioaccumulate in aquatic food webs, in contrast to many conventional pollutants. The presence of these in large numbers has led to rising concern due to their potential effects on ecosystem integrity, biodiversity and environmental sustainability. Over the last 10 years, there has been a dramatic growth of emerging contaminants in the aquatic environment, and the complexity of the pollution has significantly increased over this period. There are also a number of compounds which are not routinely monitored and are poorly regulated, even when there is evidence of biological activity in non-target species [2]. The occurrence of organic micropollutants has been documented in rivers, lakes, estuaries and coastal waters around the world, and requires a comprehensive ecological assessment of the problem [3]. The ongoing industrialisation, population growth and increasing production of chemicals are also adding to the levels of contaminant inputs, adding to the challenges for the environmental management and pollution control strategies [4]. Aquatic organisms may experience a variety of biochemical and physiological responses to exposure to emerging contaminants. These responses can be the first measurable signs of environmental stress, and they are valuable insights into the toxicity of contaminants before impacts at the population level. Physiological adaptation, alteration of metabolism, detoxification mechanisms and responses to oxidative stress are important biochemical mechanisms to determine the sensitivity of organisms to contamination exposure [5]. Hence, the assessment of biochemical responses is now an important aspect of aquatic toxicology and ecological risk assessment.

Numerous studies have shown that many emerging contaminants seem to have endocrine-disrupting properties that are capable of disrupting the endocrine regulation of aquatic organisms. These disturbances may impact growth, reproduction, development and behavioral processes, and thus lead to changes in individual fitness and population stability [6]. Besides endocrine effects, emerging pollutants have also been linked to DNA damage and mutagenic responses, possibly impacting genetic integrity and ecosystem resilience in the long-term [7]. Oxidative stress has also been identified as a major pathway for the toxicity of contaminants and affects both cellular function and antioxidant defenses, and physiological performance in fish and other aquatic species [8]. Two of the largest groups of contaminants that have been best studied are the plastic-associated contaminants and the endocrine-disrupting chemicals. Endocrine-disrupting chemicals related to plastic have been shown to disrupt hormone pathways and reproductive processes in many aquatic organisms [9]. Also, pesticides and preservative compounds found in aquatic systems were found to induce toxic effects during sensitive developmental stages, with possible impact on the survival and growth curve of an organism throughout its life cycle [10]. Overall, these observations indicate biochemical pathways are important pathways through which emerging contaminants affect ecosystem health.

While much of the previous work has contributed to the understanding of the toxicity of contaminants, the literature is still largely limited to individual chemicals, species and isolated biological endpoints. Although these studies are helpful for gaining mechanistic information, they do not always enable generalisation across contaminant classes and responses. As the number and complexity of contaminants and biological endpoints have grown, there is now a need for integrative analytical approaches that can help to synthesize complex ecotoxicological datasets and to inform on emergent patterns of toxicity responses [11]. In recent years, multivariate statistical approaches have been valuable tools in studying environmental data sets because they can uncover dominant variables within the data, hidden relationships, and sources of variation in complex environmental systems [12]. They have been widely used in environmental assessment projects, especially to assess contaminant distributions and ecological responses from a variety of data sources [13].

Although there is emerging research focusing on emerging contaminants, there are still critical knowledge gaps about the relative toxicity of the main groups of contaminants and the biochemical effects in aquatic organisms. There have been a few studies that have jointly considered thresholds, life stage sensitivity, contaminant type, and response categories in one analysis. Therefore, the relative significance and contribution of various groups of contaminants and biological endpoints in determining ecotoxicological effects are only partly understood. In addition, little research has used multivariate and statistical integrated assessment tools to assess general trends of biochemical responses to contaminants and their implication for ecosystem health and sustainability.

In this context, the present study aims to investigate the biochemical responses of aquatic organisms in the presence of emerging environmental contaminants, with the aid of an extensive ecotoxicological database. This study examines the frequency distribution of contaminant categories and biological response types, the relative toxicity thresholds among each of the different contaminant classes, among different life stages and among biochemical endpoints, and multivariate relationships between exposure characteristics and toxicity indicators. The study helps build a general understanding of the effects of emerging contaminants on aquatic ecosystems and is used for environmental monitoring, ecological risk assessment and sustainable ecosystem management, and will help identify key patterns of contaminant sensitivity and biological response.

2. METHODOLOGY

2.1 Research Design

For this study, a quantitative secondary data analysis design was used to explore the biochemical changes of aquatic organisms in the presence of emerging environmental contaminants. The analysis aimed to assess toxicity thresholds and to look for trends in biological effects due to contaminants in the different categories of fish, contaminant classes, life stages, and responses. To assess differences in toxicity sensitivity and to investigate wider relationships between contaminant properties and biological endpoints, statistical and multivariate techniques were employed.

2.2 Data Source

Data employed in the study were from publicly available ecotoxicological data [14]. The dataset includes experimental exposure history and biological effects of contaminants for fish species, and details of contaminant identity, experimental exposure conditions, life stage, experimentally determined toxicity thresholds, and biological response observed under the experimental conditions. The dataset is a comprehensive collection of ecotoxicological evidence, which is useful to understand the impacts of COCs on aquatic organisms.

2.3 Data Preparation and Screening

The data underwent a systematic data cleaning process. The names of variables were standardized, duplicate header rows were eliminated, and records were checked for completeness. Relevant variables to the goals of the study were kept, such as contaminant category, species information, life stage, exposure duration, biochemical response category, and toxicity threshold measures. Since toxicity thresholds were provided in a variety of endpoint measurements, the lowest observed adverse effect concentration (LOAEC) was chosen for use as the primary toxicity indicator because it is the most readily available across the records. Observations with no results of LOAEC were not used in quantitative analyses. In order to make the categories more uniform, contaminant classifications and biochemical response categories were harmonized (combining equivalent terms and correcting minor inaccuracies in the names). After screening and cleaning, 491 observations were left for statistical analysis.

2.4 Statistical Analysis

Characteristics of the dataset, contaminant distribution, biochemical response categories and toxic thresholds were described. The frequencies, percentages, medians and ranges were computed to describe the data set. There was a great deal of variability in scores for LOAEC, and thus the scores were log transformed (to base 10) before any inferential

analyses. The Kruskal – Wallis test was used to compare the differences in toxicity thresholds between categories of contaminant, life stage, and biochemical response due to non-normality of data. Epsilon-squared (ϵ^2) was used to measure the magnitude of the observed differences, thus calculating effect sizes. Pairwise comparisons were done using Mann–Whitney U tests (with multiple comparison correction) for significant differences. Median $\log_{10}$ LOAEC values and 95% bootstrap confidence intervals were estimated for each contaminant group to make them comparable across groups for toxicity thresholds. The method enabled the visualizing the central tendency and uncertainty of toxicity thresholds without assuming a distribution.

2.5 Multivariate Analysis

The multivariate structure of the data was analyzed and variables that contributed most to the observed variables were identified by using Principal Component Analysis (PCA). The categorical variables were transformed into binary indicator variables before analysis, along with continuous variables such as duration of exposure and $\log_{10}$ LOAEC. Data were transformed so that all of them were on the same scale of measurement. Evaluation of the PCA results was done based on the eigenvalue, proportion of explained variance and the component loading. The first two principal components were interpreted since they explained the highest percentage of the variation, and they indicated the predominant contaminant and biological responses in the data set.

3. RESULT

3.1 Dataset Characteristics and Distribution of Contaminants and Biochemical Responses

After screening the records that had available toxicity threshold data, 491 observations were included for quantitative analysis. These observations consisted of 37 different contaminants from 10 different contaminant categories, 81 species of fish, and 21 biochemical response categories. The median LOAEC was 9.0, and the reported LOAEC values ranged over a decade of orders of magnitude (from 1.0×10^7 to 2528.3). The broad range indicates significant differences in the sensitivity of aquatic organisms and biological endpoints to contaminants. The overall characteristics of the analytical data set are summarized in Table 1.

Table 1. Summary characteristics of the dataset used for toxicity threshold analyses

Characteristic	Value
Total observations	491
Unique chemicals	37
Contaminant categories	10
Fish species	81
Biochemical response categories	21
Median LOAEC	9.0
Minimum LOAEC	1.0×10^{-7}
Maximum LOAEC	2528.3

Nearly half of the observations (46.23%) were dominated by hormone-related contaminants, followed by industrial compounds (12.22%), phthalates (11.20%) and PFAS compounds (9.37%). The most commonly reported biological endpoints were development (18.13%), endocrine (16.09%) and physiological (15.68%). All three of these groups of endpoints accounted for about half of all documented biochemical responses. The distribution of contaminant categories present in the data is as follows (Figure 1A). The distribution of biochemical response categories by the aquatic organisms is illustrated in Figure 1B.

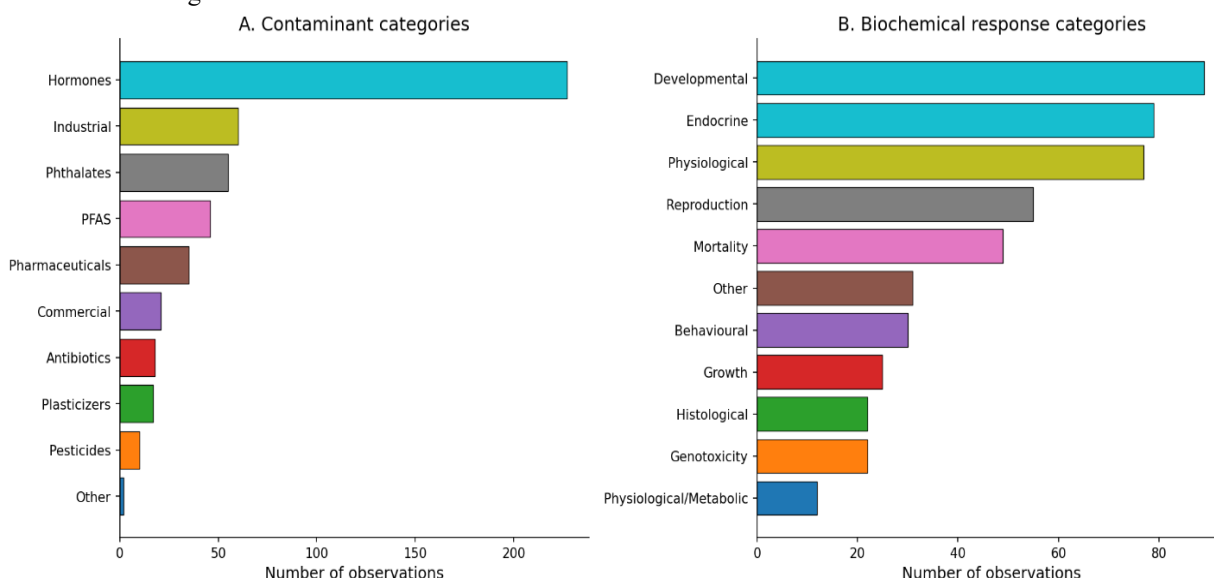


Figure 1. Distribution of contaminant categories and biochemical response categories. (A) Frequency distribution of contaminant categories represented in the analytical dataset. (B) Frequency distribution of biochemical response categories reported across aquatic organisms exposed to emerging contaminants.

3.2 Differences in Toxicity Thresholds among Contaminant Categories

There were significant differences in toxicity thresholds between contaminant categories. Median $\log_{10}$ LOAEC values were highest for commercial compounds and pharmaceutical contaminants, suggesting adverse biochemical effects generally at relatively high concentrations. PFAS compounds and plasticizers, on the other hand, had the lowest median toxicity thresholds, indicating a higher biological sensitivity to these groups of contaminants. Significant differences in $\log_{10}$ LOAEC values from the Kruskal–Wallis test were found between contaminant categories ($H = 39.49$, $p < 0.001$). While statistically significant, the effect was moderate ($\epsilon^2 = 0.0656$), suggesting that contaminant classification accounted for a small but substantial percentage of the variability in toxicity thresholds. Median $\log_{10}$ LOAEC values with 95% percent-ile (95% percentile bootstrapped confidence intervals) are shown in Figure 2 for each category of contaminants. Major contaminant categories with median $\log_{10}$ LOAEC values and 95% Bootstrap confidence intervals. The dashed line shows the overall median toxicity threshold for all observations. Pairwise post-hoc analyses also showed that commercial compounds and hormones were significantly different from antibiotics, phthalates, PFAS compounds, and industrial compounds; and that hormones and industrial compounds were significantly different from each other. The toxicities of these classes of contaminants are seen to vary significantly with concentrations needed to cause adverse biochemical responses.

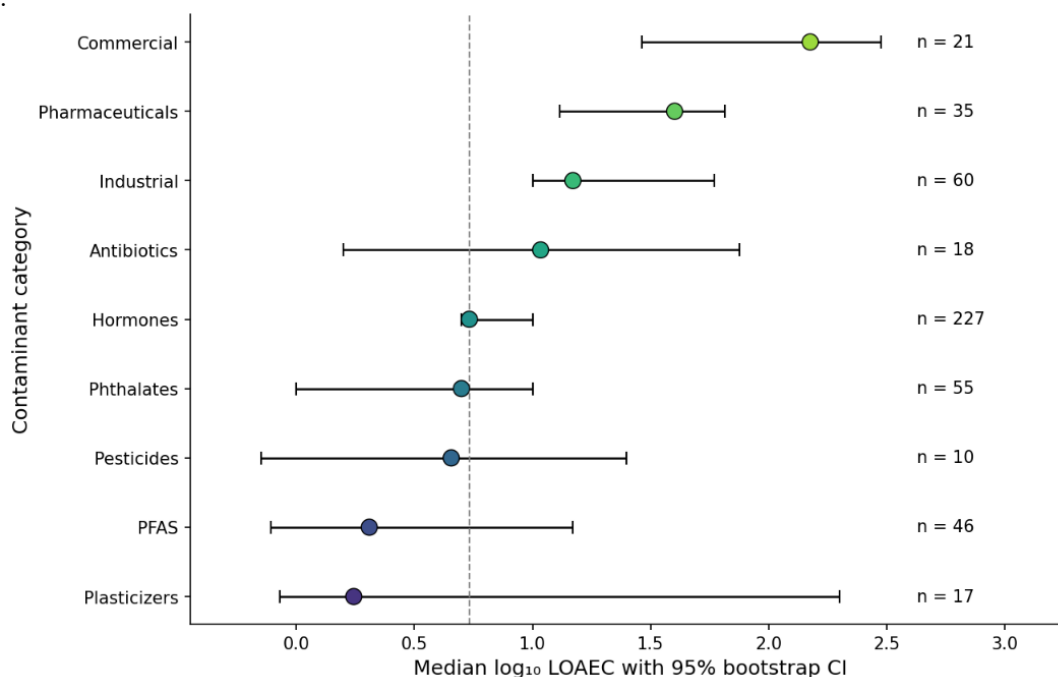


Figure 2. Median toxicity thresholds across contaminant categories.

3.3 Statistical Comparisons of Toxicity Thresholds Across Biological Factors

Comparative analyses were made for differences in toxicity thresholds across contaminant categories, life stages, and biochemical response categories. The Kruskal–Wallis test showed that there were significant differences in toxicity thresholds between life stage ($H = 15.69$, $p = 0.001$), contaminant category ($H = 39.49$, $p < 0.001$), and biochemical response category ($H = 28.76$, $p < 0.001$). Effect sizes were in the small to moderate range, indicating these factors were not the sole cause of toxicity variability, but they did play a role. The results of hypothesis testing for the three important analytic comparisons are presented in Table 2. Life-stage comparisons revealed that the sensitivity of fish was higher at the embryonic, juvenile and larval stages than the adult stage, thus suggesting that adults have higher toxicity thresholds. Also, the biochemical response categories showed markedly different response thresholds, with genotoxic, physiological, and endocrine responses being more likely to be seen at higher concentrations than developmental and histological responses.

Table 2. Kruskal–Wallis tests evaluating differences in toxicity thresholds

Hypothesis	Comparison	Test	H statistic	p-value	Epsilon-squared	Decision
H1	LOAEC across contaminant categories	Kruskal–Wallis	39.4903	<0.001	0.0656	Significant
H2	LOAEC across life stages	Kruskal–Wallis	15.6909	0.001	0.0280	Significant
H3	LOAEC across biochemical response categories	Kruskal–Wallis	28.7581	<0.001	0.0473	Significant

3.4 Multivariate Patterns of Contaminant Exposure and Biological Responses

A multivariate analysis of toxicity thresholds, contaminant category, duration of exposure and biochemical response categories was performed using principal component analysis (PCA). The first two PCs accounted for 10.1% and 8.2% of the total variance, respectively, for a total of 18.3% of the variance in the data set. The relatively low cumulative variance

explained by the first two components suggests significant complexity in the relationships between contaminants and toxicity, and that there are multiple interacting factors that give rise to observed toxicity patterns. However, several variables that were dominant in influencing the overall variability were identified by the PCA. For each of the principal variables, the variation along PC1 is shown in Figures 3A through 3F. The main variables causing variation along PC2 are summarized in Figure 3B. The hormone contaminants, exposure duration and endocrine responses were the major positive contributors to PC1, while mortality, industrial contaminants and genotoxic responses were the major negative contributors. Developmental responses and plasticizers positively influenced the PC2, whereas endocrine responses, physiological responses, hormone contaminants, and toxicity thresholds negatively influenced the PC2. Based on these patterns, it is clear that developmental and endocrine pathways are key but opposing aspects of biochemistry induced by contaminants in aquatic organisms.

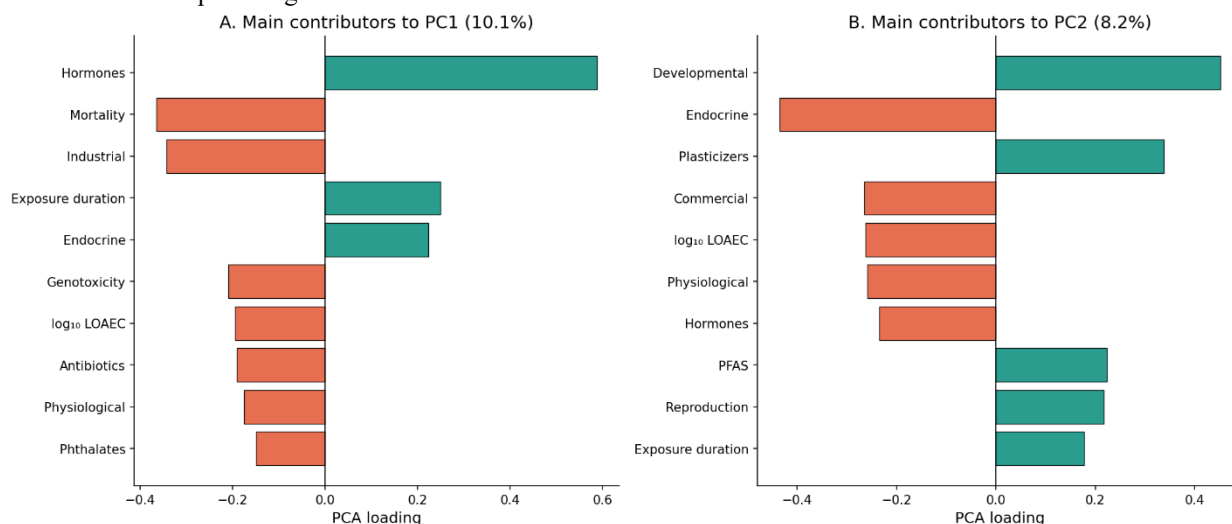


Figure 3. Principal component loadings describing multivariate variability in toxicity responses. (A) Major positive and negative contributors to Principal Component 1 (PC1), explaining 10.1% of total variance. (B) Major positive and negative contributors to Principal Component 2 (PC2), explaining 8.2% of total variance.

4. DISCUSSION

The results showed that there is no common pattern for the biochemical effects in aquatic organisms exposed to emerging environmental contaminants, based on contaminant class, life stage or biological endpoint. Almost half of the analytical observations were from hormone-related contaminants. The pattern indicates that endocrine active substances continue to be a key area of interest within aquatic ecotoxicology, as they have the ability to cause biological effects at low concentrations. The high prevalence of developmental, endocrine and physiological endpoints also points to the fact that exposure to contaminants can cause effects that are not necessarily lethal, but instead result in disruption of function, such as growth, endocrinology, metabolism and organismal function. The large range of log₁₀ LOAECs for the contaminant classes suggests that the different classes of emerging contaminants have various abilities to elicit adverse biochemical responses. The median toxicity thresholds were relatively low for PFAS compounds and plasticizers, suggesting possible lower toxicity levels for these groups of contaminants than for others. The patterns of the commercial compounds and pharmaceuticals did have higher median thresholds; however, the meaning of these results should be considered in the context of endpoint type, exposure duration, test species and number of observations per test group. The moderate effect size for the contaminant category indicates that the toxicity is influenced by chemical class but not by the chemical alone. There were also differences between the different life stages. Generally, toxicity thresholds of adults were higher while those of larvae and early developmental stages were lower. This reflects the potential for greater susceptibility of early life stages due to immature detoxification mechanisms, organ maturation, immune system deficiency and endocrine regulation. Ecologically, a reduced developmental threshold of toxicity is significant because developmental impairment may lead to impaired recruitment, change the probability of survival, and impact population persistence, even if adults are not as sensitive. The results of the biochemical response categories also support the conclusion that the effects of contaminants are endpoint-specific. Some developmental, histological and reproductive responses were observed at lower threshold levels compared to other physiological and genotoxic responses. The patterns indicate that early structural and functional disturbances can occur prior to the discovery of more severe and/or highly specific damage. The PCA results offered an exploratory multivariate view but did not offer a good separation of the groups. The associations of PC1 were primarily with hormone contaminants and endocrine response, whereas the associations of PC2 were primarily with developmental responses, plasticizers, PFAS and endocrine-related variables. The low explained variance supports the notion that contaminant response is complicated and multifaceted, involving multiple biological and exposure factors.

The predominance of hormone-related chemicals is reflective of the ubiquity of these compounds in aquatic environments and their ability to cause ecological effects through bioaccumulation and hormonal effects on aquatic organisms [15]. It is also in line with the results from freshwater species that have shown that the activity of hormones is disrupted by xenobiotics at sublethal concentrations, affecting reproduction, growth and developmental processes [16]. The increased sensitivity of the early life stages is consistent with the general knowledge that aquatic organisms have complex life cycles with different physiological constraints and ecological vulnerabilities at different stages of their development [17]. The importance of biological traits like life-history stage, reproductive strategy, mobility and physiological tolerance on

species' vulnerability to human pressures is also emphasized in trait-based vulnerability frameworks [18]. In view of this, the present finding of the toxicity thresholds that cannot be understood apart from the organismal development is supported by these perspectives.

An important reason for the prominence of physiological responses in the dataset is evidence that contaminants of emerging concern can affect the physiology of fish and invertebrates, including alterations to metabolism, stress regulation, immune function and organismal performance [19]. Oxidative stress is also considered a key mechanism underlying the cellular injury and organismal dysfunction in aquatic and terrestrial organisms, as a consequence of pollutant exposure [20]. The present results do not directly quantify oxidative biomarkers, but the patterns of physiological or developmental endpoints are consistent with biochemical disruption being a major pathway of effects from contaminants. Studies that report on alteration of fish tissue parameters related to water quality degradation show this relationship between pollutant exposure and altered fish tissue parameters [21]. Studies that have shown bioaccumulation and toxicity of pharmaceuticals like diclofenac can be altered by biotransformation, further emphasize the importance of contaminant-specific metabolism in understanding toxicity thresholds [22]. These results contribute to an understanding of the variable patterns of toxicity within and across contaminant classes and the need for a single exposure-response model for all emerging contaminants.

The findings are relevant for assessing the health of aquatic ecosystems, as biochemical and developmental responses can be indicators of aquatic ecosystem stress. Subchronic and chronic exposure may cause effects on endocrine, developmental, reproductive and physiological functions that can impact survival, reproductive capacity and population structure in the future. The use of chemical occurrence as the sole basis for regulatory and monitoring programmes should be evaluated by biological sensitivity and severity of endpoints for certain groups of contaminants. The results were supportive of a risk-based approach for contaminant prioritization, which would be beneficial for sustainability. PFAS, plasticizers, hormone-like chemicals and other biologically active chemical substances should be monitored continuously as exposure at sub-lethal levels could lead to chronic exposure to the environment. Toxicity thresholds, sensitive life stages, and biochemical response categories should be incorporated into the monitoring program to enhance its ability to detect those contaminants that are ecologically more relevant than others. This integration can help to better manage wastewater, inform contaminant screening and inform ecosystem-based decision making.

Some caveats are called for. Secondary data from various studies with different experimental designs, exposure times, species and endpoint definitions were used for the analysis. LOAEC was chosen due to its availability; other endpoints, like NOEC, LC50 or reported measured concentrations of a biomarker, were not as consistently reported. The number of observations was fewer for some categories of contaminants or for some responses, which may affect comparisons between categories. Standardized information on toxicity thresholds, exposure units, life stage information, and biochemical endpoints should be a priority for future studies. Complementary studies combining enzyme, oxidative stress markers, transcriptomic responses to enzyme activity and metabolomic profile would further support mechanistic interpretation. Mixture toxicity and multiple-stressor studies should also be conducted due to the fact that in natural environments, aquatic organisms are not usually exposed to only one stressor. The wider incorporation of taxa beyond fish would yield a greater understanding of the sensitivity of the ecosystems to emerging environmental contaminants.

5. CONCLUSION

The biochemical effects of emerging environmental contaminants were measurable and variable, and differed among contaminant classes, life stages and biochemistry endpoints. The most representative contaminants were the hormones, and the most common biological response was developmental, endocrine and physiological responses. Log₁₀ LOAEC values for contaminants were found to be significantly different, indicating that the LOAEC values for contaminants are dependent on contaminant class; the median LOAEC values for PFAS and plasticizers were relatively low and suggested a higher potential for biological sensitivity. Lower toxicity thresholds were recorded for early life stages than adults, suggesting that embryos, juveniles and larvae are more susceptible to exposures to contaminants. Further differences between biochemical response categories also indicate the relevance of developmental, histological and reproductive endpoints as indicators of stress caused by contaminants. The PCA results confirmed that contaminant responses were complex, with significant patterns of variation related to hormones and development. The results demonstrate the advantages of combining the toxicity thresholds, life stage sensitivity and biochemical response categories in aquatic risk assessment. Improved and standardized ecotoxicological reporting and enhanced monitoring of contaminants will help to better identify contaminants of high concern and the effective management of aquatic ecosystems.

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