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Occurrence, bioaccumulation, and ecological risk assessment of contaminants of emerging concern using native and invasive species as biomonitors

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A B S T R A C T

Traditionally, contaminants of emerging concern (CECs) monitoring have focused on assessing their occurrence in abiotic compartments and in native fish species. The use of non-native species in environmental quality studies has recently proven to be a key tool for evaluating the CECs exposure and their ecological risks. In this study, the use of different native and invasive species was proposed to assess the environmental quality of a coastal Mediterranean wetland. A biomonitoring assessment was conducted at ten locations within the Albufera Natural Park (Spain), including irrigation channels, the lake, and the artificial wetland. The bioaccumulation of 171 CECs was evaluated in the Asian clam, the American red swamp crayfish, and the pumpkinseed sunfish, which are primary invasive species in this ecosystem. Furthermore, a comparative analysis was done with the native clam to verify whether invasive species could provide equivalent information. A total of 35 CECs were detected in at least one of the species analyzed. The Asian clam exhibited the highest number of detected compounds (23), as well as the highest chemical concentrations, particularly for pharmaceuticals. The ecological risk assessment performed with internal concentrations of CECs also pointed at the Asian clam as the most suitable species for chemical biomonitoring in this area. The compounds that had the highest contribution to the calculated ecological risk were sertraline, fluoxetine, terbuthylazine, caffeine, and oseltamivir. At most exposure sites HI values revealed high risk, indicating strong pressure from mixtures of CEs for both native and invasive species. This study shows that the analysis of chemical concentrations in invasive species can be considered a complementary tool to determine the ecological status of coastal wetlands.

1. Introduction

Freshwater ecosystems, including wetlands, are continuously exposed to contaminants of emerging concern (CECs) such as organophosphate flame retardants (OPFRs), per- and poly-fluoroalkyl substances (PFAS), pesticides, and pharmaceutically active compounds (PhACs) [1]. The biomonitoring of CECs in selected aquatic species provides crucial insights into the levels of contaminant exposure and bioaccumulation in aquatic biota, which can be better linked to chemical-related effects in aquatic ecosystems [2–5]. Invasive aquatic species could serve as effective bioindicators due to their greater tolerance to environmental stress and their capacity to bioaccumulate contaminants, as in the case of zebra mussels [6]. Using invasive species in biomonitoring can also reduce the pressure on native species, as invasive populations are typically larger and more abundant so that their use in environmental quality assessments minimizes ecosystem

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disturbances [7,8]. However, most biomonitoring studies of CECs in freshwater ecosystems have focused on the assessment of a narrow range of compounds in native fish species, neglecting the value of invasive species for environmental quality studies [9] [10–12].

L'Albufera Natural Park (ANP) in Valencia, Spain, is a strategic aquatic ecosystem that faces several anthropogenic pressures [1], including the colonization by invasive species such as the American crayfish, *Procambarus clarkii* (native to northeastern Mexico and the southern U.S.) [13], the pumpkinseed sunfish, *Lepomis gibbosus* (an ornamental fish introduced for sport fishing from eastern North America) [14], and the Asian clam, *Corbicula fluminea* (one of the world's most widespread invasive clams from East Asia) [15]. These species compete with endangered endemic species, such as *Anodonta cygnea*, a clam whose abundance has declined significantly in Mediterranean coastal wetlands and that currently is under special protection [16].

Due to the proximity of the ANP to the city of Valencia and the various economic activities in the region, the Park is continuously subjected to significant pressure from CECs, including OPFRs and PFAS from urban and industrial sources; pesticides from rice and citrus cultivation in the vicinity of the lake, and PhACs from WWTPs effluents that are commonly used for rice field irrigation [17–19]. Despite the number of studies pointing at complex CEC mixtures as one of the main drivers of biodiversity loss in the aquatic ecosystems [20] including the ANP [17], there have been no studies aimed at assessing their bioaccumulation potential and their ecotoxicological risks in sentinel species representative of this ecosystem.

In this study we comparatively assessed the bioaccumulation potential of CECs in three common invasive species, and a protected native species representative of the ANP and determined their capacity to be used as sentinel organisms to assess the environmental quality of Mediterranean coastal wetlands. The specific objectives of this study were: i) to evaluate the occurrence and bioaccumulation of 49 pesticides, 86 PhACs, 36 industrial compounds including 11 OPFRs, 20 PFAS, and 5 other industrial compounds in different strategic areas of the ANP, ii) to determine differences in CECs bioaccumulation potential between invasive and native species, and iii) to perform an ecological risk assessment using invasive and native species as biomonitors of ecosystem quality.

2. Materials and methods

2.1. Chemicals and reagents

The complete list of reference standards used in this study can be found in Table S1, while its provenance and their isotopically labeled standards (IS) are detailed in ***Text S1.*** The solvents and materials used for the extraction method and chromatographic separation are specified in ***Text S2*** and ***Text S3***. Calibration curves were generated using standard mixtures prepared through serial dilutions from a 2 μg mL^{-1} stock solution. A matrix-matched calibration curve was created by spiking the extracts to achieve ten concentration levels, ranging from 0.1 to 500 ng g^{-1} (wet weight, w.w.).

2.2. Study area and sampling design

The study was carried out in the ANP (Valencia, Spain), a protected ecosystem since 1986 due to its ecological value as a key habitat for threatened endemic and migratory species [18]. The area spans 211.2 km^2, including wetlands, lagoons, marshes, dunes, and rice fields [19], with the Albufera lake (23.7 km^2), at its center, surrounded by rice fields (223 km^2), as the dominant crop [19,21]. The ANP features an extensive network of irrigation ditches and channels that supply water to the rice fields and transport drainage waters to the Albufera lake [18]. The ANP receives effluents from wastewater treatment plants (WWTPs), such as the Pinedo 2 WWTP, which are used for irrigation of rice fields in the northern part of the ANP and that end up in the lake [17,21]. The middle and southern parts are irrigated with waters from the Turia and Júcar River basins, transported through a 59.7 km network of artificial channels. Overall, rice fields contribute about 40 % of the ANP total water flow [21].

The specimens used in the study were captured from ditches and ponds within the ANP, and local experimental facilities. After capture, they underwent a depuration period of 14 days in 26 L tanks with a closed-circuit system of clean water and a filtration module at El Palmar Fish Research Center (Valencian Government). Some individuals from each species were sacrificed post-depuration and used as reference biological material.

To assess the CEC bioaccumulation potential of each species, exposures were conducted at ten sampling sites within the ANP, covering three distinct habitats. Two sites were located in the lake (one in the north and one in the south), and two in the artificial wetland that receives effluents from the Albufera Sur WWTP (one at the entry and one at the exit). Additionally, four sites were selected at the irrigation and drainage channels of the rice fields (channels of Overa, Alqueresia, Font Nova, and Comú), along with two points at channels that also receive effluents from WWTPs (channels of Campets and Tancaeta). Two control points were included to monitor species mortality: one at a fish research Centre of Natural Park (Control 1) and another at the channel Real del Júcar, which brings clean waters for orchards and rice field irrigation (Control 2) (Fig. 1). The physicochemical parameters of the water measured at the different exposure sites and control points are shown in Table S2.

Cages containing individuals of each species were placed at the field on September 21, 2020, and were exposed to the surface water for 14 days. *C. fluminea* and *A. cygnea* were placed in three cages per site with a sandy substrate inside the cage, with eight *C. fluminea* and one *A. cygnea* per cage. *P. clarki* were placed in three cages per site, with each cage containing four individuals, some local vegetation and PVC tubes serving as refuges. Four cages of *L. gibbosus* were placed per site, with each cage containing one individual. The cages allowed the water to circulate through them and food to be obtained from the exposure medium. Examples of the cages used are shown in Fig. S1. The cages were tied next to each other and sunk in the water column at an approximate distance of 30–40 cm from the sediment. At the end of the exposure period, the fish were sacrificed by asphyxiation, while the other species were sacrificed via freezing. ***Text S4*** briefly describes the pretreatment of the biotic samples and surface water sampled at each exposure site.

2.3. Extraction method

The method for extracting CECs from invasive and native species has been carefully detailed in a previous study [22] and is briefly described in ***Text S5***. Surface water samples were extracted by Solid Phase Extraction (SPE) according to the method described by [18], which is briefly summarized in ***Text S6***.

2.4. Quantitative analysis by LC-HRMS/MS

Details about the chromatographic separation, mobile phases used for ionization modes, elution gradient, source conditions, and other parameters related to the HRMS/MS acquisition method for

Fig. 1. Map showing the locations where CECs were monitored and where the cages were located (Control sites, channels, lake, artificial wetland). The locations are presented in higher resolution on the right-hand side of the figure.

tissue sample analysis are summarized in ***Text S7*** [22]. Information regarding the chromatographic separation and analytical method for each group of compounds analyzed in the surface water were previously published [18,23].

2.5. Quality control

The survival rate of the exposed species was 98 %, including the ten sampling sites and the two control sites selected for mortality monitoring. During acquisition, matrix blanks spiked with reference standards at a concentration of 50 ng mL^{-1} were analyzed after every five samples as a quality control to assess method performance. Additionally, solvent blanks (methanol) were analyzed to confirm the absence of any carryover in the column. The R^2 for all compounds was greater than 0.99. The recoveries, linearity, and limits of detection and quantification of the method for each compound and per matrix were previously detailed [22].

2.6. Data analysis

For the analysis of CECs in the species, median concentrations for each compound were calculated for compounds detected in at least two of the triplicate samples per species (75 %) and present in at least one sampling site. For samples meeting these criteria, values below the limit of detection (LOD) or limit of quantification (LOQ) were replaced with LOD/2 or LOQ/2 as appropriate. Additionally, a comparative analysis using the Kaplan-Meier non-parametric method as implemented in ProUCL 5.2 (EPA, USA) was conducted to assess the potential bias introduced by substitution methods. The comparison showed no significant differences in the calculated medians, likely due to the low proportion and magnitude of censored values. Nevertheless, we support and encourage the broader use of non-parametric methods such as Kaplan-Meier in environmental studies, especially when dealing with datasets with higher levels of censoring as proposed by [24,25].

To estimate the accumulated concentration over the 14-day exposure period, the concentrations measured in the blanks analyzed after the depuration process were subtracted. Most blanks showed undetectable concentrations, except for: TDBPP (0.60 ng g^{-1}), and caffeine (94.2 ng g^{-1}) in *P. clarkii*; caffeine (0.5 ng g^{-1}), carbafuran -3-hydroxy (<LOQ), TDBPP (0.6 ng g^{-1}), TMPP (1.3 ng g^{-1}), cotinine (21.4 ng g^{-1}), fluoxetine (0.3 ng g^{-1}), o-desmethylvenlafaxine (0.1 ng g^{-1}), salbutamol (6.4 ng g^{-1}), tramadol (1.2 ng g^{-1}) and venlafaxine (0.2 ng g^{-1}) in *C. fluminea*; TDBPP (0.5 ng g^{-1}), TPP (1.1 ng g^{-1}), bisphenol A (0.5 ng g^{-1}), salbutamol (0.4 ng g^{-1}), and tramadol (0.1 ng g^{-1}) in *L. gibbosus*; TDBPP (2.1 ng g^{-1}), TMPP (0.9 ng g^{-1}), benzotriazole (0.3 ng g^{-1}), citalopram (0.6 ng g^{-1}), cotinine (10.2 ng g^{-1}), fluoxetine (0.6 ng g^{-1}), methadone (18.1 ng g^{-1}), and tramadol (0.1 ng g^{-1}) in *A. cygnea*.

Statistical analyses were performed using Origin 2022 (OriginLab Corp., USA). Values < LOQ were replaced with LOQ/2. Since the Shapiro-Wilk test revealed a non-normal distribution of the data (p-value <0.05), non-parametric tests were used. Specifically, the Kruskal-Wallis test was applied in a pairwise manner between species to assess significant differences in the summed medians of CECs families. For each family, concentrations of individual compounds were grouped, and their medians summed by species and exposure site.

To relate the concentrations detected in surface water with those found in the monitored species and to assess differences among sampling sites, bioaccumulation factors (BAFs) were calculated by dividing the mean concentration per compound and species at each site by the mean concentration of the same compound in surface water, according to Eq. (1). The mean concentrations for surface water were based on the two measurements taken at the start of the exposure period and at the end of the exposure period (i.e., with a 14-d time interval), while the internal concentrations in the monitored species corresponded to those measured at the end of the exposure period.

$$BAF = \frac{Mean\ concentration\ C_i\ Sp_i\left(ng\ g^{-1}\right)}{Mean\ concentration\ surface\ water\ Site_i\ \left(ng\ L^{-1}\right)}\ x\ 1000 \quad \text{Eq. (1)}$$

Where C_i represents each compound, Sp_i each species, and $Site_i$ each sampling site.

Finally, to assess the risks of the measured concentrations for the different species, risk quotients (RQ) were calculated by comparing the measured environmental concentration (MEC) with the predicted no-effect concentration (PNEC) proposed by the NORMAN ecotoxicology database [26] according to Eq. (2). The database includes specific PNECs for internal concentrations in freshwater fish and mollusks, but none are available for crustaceans. Therefore, in this study, the PNECs for mollusks were also applied to the crab *P. clarkii*, as both species crabs and clams, fall under the category of aquatic invertebrates, ensuring similar reference levels.

$$RQ = \frac{MEC}{PNEC} \quad \text{Eq. (2)}$$

The RQ calculation was performed by compound and species at each of the exposure sites. The results were classified as posing high risk ($RQ \geq 1$), medium risk ($0.1 \leq RQ < 1$), and minimal risk ($RQ < 0.1$). Finally, a Hazard Index was calculated as the sum of the RQs for a given species in a given sampling site, assuming additive effects of the measured contaminant mixture.

3. Results and discussion

3.1. Occurrence of CECs in surface waters and monitoring species

In total, the presence of 63 PhACs, 26 pesticides, 14 PFAS, 7 OPFRs and 2 other industrial compounds was confirmed in at least one of the surface water samples (Table S3). The sampling site located at the inlet of the artificial wetland (Wet. Entry) had the highest accumulated mean concentration of CECs (18343 ng L^{-1}), followed by Camptes (5316 ng L^{-1}). The high concentrations at Wet. Entry was expected as it receives effluents from the Albufera Sur WWTP [17], which also influences Camptes due to its proximity. The detection of compounds varied across species. Out of the 171 compounds studied, 35 were detected in the monitoring species. Fig. 2 shows the species in which each of these compounds was detected. The species with the highest number of detected compounds (23) and the highest reported concentrations was the clam *C. fluminea* (Fig. 3).

Other Industrial compounds: In surface water, the highest concentrations were observed for benzotriazole, categorized under other industrial compounds, with levels ranging from 19.9 to 12650 ng L^{-1} (Fig. S2). Among the compounds included in this category, it was the only one detected in all species. The highest median concentration was reported in *L. gibbosus*, with 70.2 ng g^{-1} in Wet Entry. In *C. fluminea*, was detected at 57.7 ng g^{-1} at Alqueresia, while in *A. cygnea*, it was 48.9 ng g^{-1} at Camptes, and in *P. clarkii*, the highest median concentration was 32.2 ng g^{-1} at Wet Entry.

PhACs: Bezafibrate showed the highest mean concentration in surface water, and it was detected at all exposure sites at levels ranging from 83.0 to 2741 ng L^{-1}, followed by ibuprofen, which ranged from 49.8 to 657 ng L^{-1}. These concentrations exceed those reported previously [27], where bezafibrate levels were between 1.0 and 79.0 ng L^{-1}, and ibuprofen levels ranged from 20.0 to 217 ng L^{-1} in the same study area. In contrast, the concentrations of atenolol (0.2–4.3 ng L^{-1}) and tramadol (3.1–377 ng L^{-1}) detected in our study were lower than those previously reported, which were 52–221 ng L^{-1} for atenolol and 100–1260 ng L^{-1} for tramadol [27].

For the species, the highest concentration was for sertraline (413 ng g^{-1}) at the Alqueresia site, followed by Wet Entry (344 ng g^{-1}) in *C. fluminea.* Oseltamivir had the highest detection frequency in *C. fluminea*, found at all exposure sites except Lake South, with concentrations ranging from 27.6 ng g^{-1} (Lake North) to 134 ng g^{-1} (Campets). The site with the highest cumulative median concentration for this species was Wet. Entry (1032 ng g^{-1}), followed by Alqueresia (618 ng g^{-1}).

Antidepressants such as sertraline and fluoxetine have previously been reported in *C. fluminea* at concentrations lower than those found in this study. Previous studies reported levels ranging from 56 to 226 ng g^{-1} for sertraline and from 5.5 to 12.0 ng g^{-1} for fluoxetine [28,29]. Although the detection frequency in these studies was higher, in the present study, the presence of these compounds is primarily associated with the Wet. Entry exposure site, where higher contributions of effluents from the WWTP are received, which may explain the differences observed.

PFAS: PFOS had the highest mean concentrations in water: 10.2 ng L^{-1} at Lake North and 8.1 ng L^{-1} at Lake South. The mean concentrations reported for the PFAS group in this study are lower than those previously recorded in the study area. Lorenzo et al. (2019) [30] reported mean concentrations of 31.6, 16.1, and 9.7 ng L^{-1} for PFOS, PFPeA, and PFOA, respectively; while the concentrations obtained in our study were below 5 ng L^{-1} for PFPeA and PFOA across all exposure sites.

PFAS were reported in all species and at all exposure sites, with *L. gibbosus* showing the highest detection frequency for these compounds. PFBA had the highest median concentration across all species: 69.7 ng g^{-1} in *C. fluminea*, 47.5 ng g^{-1} in *L. gibbosus*, 40.0 ng g^{-1} in *P. clarkii*, and 33.2 ng g^{-1} in *A. cygnea*. PFOS followed in *L. gibbosus* with 39.2 ng g^{-1}. The other detected PFAS were reported at concentrations below 8.0 ng g^{-1}. The presence of PFAS, such as PFBA, has previously been reported in the muscle tissue of *P. clarkii,* with concentrations <2.5 ng g^{-1} [31], which are lower than those reported in this study. In contrast, PFHxS concentrations reached up to 2.0 ng g^{-1}, slightly higher than those observed in our findings.

In crayfish from the Ebro Delta (Spain), no PFAS were detected in the muscle tissue of *P. clarkii*. However, when analyzing the whole body, including the head, concentrations of PFOS and PFOA, and, to a lesser extent, PFNA were reported, ranging from 0.1 to 0.6 ng g^{-1} [32]. Nevertheless, in ANP, a similar ecosystem, these compounds were not detected in *P. clarkii* but were found in *C. fluminea* and *L. gibbosus*, except for PFNA.

OPFRs: TClPP had the highest mean concentration in surface water, with a value of 740 ng L^{-1} at Wet Entry. The mean concentrations of OPFRs obtained in this study are higher than those previously reported, but only at the entry of constructed wetlands. Compounds such as TCEP, TDCIPP, and TCIP showed higher mean concentrations (146, 67.0, and 740 ng L^{-1} respectively), compared to 6.4, 17.4, and 70.4 ng L^{-1} reported previously [30].

TDBPP and TMPP were the compounds most frequently detected across all species. TDBPP showed the highest median concentrations in the invasive species, with values of 32.3 ng g^{-1} in *P. clarkii* and 31.9 ng g^{-1} in *L. gibbosus* at Lake South, and 32.2 ng g^{-1} in *C. fluminea* at C. Comú. The absorption of OPFRs through gills is more common than their absorption via the food chain [33]. This could explain why TDBPP and TMPP were detected in all species analyzed, regardless of their diet. Research on the presence of OPFRs in the species examined in this study is limited. However, some reports indicate the presence of compounds such as TCPP and TPP in perch, with concentrations ranging from 170 to

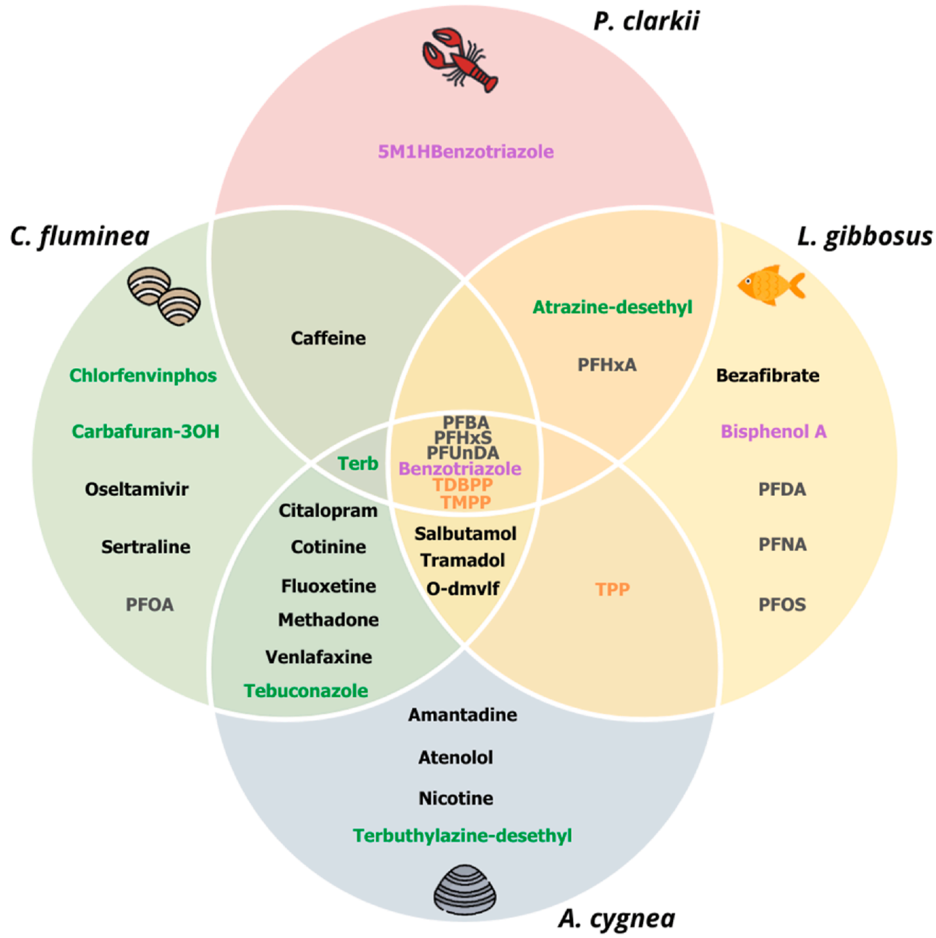

Fig. 2. *Venn diagram of the* 35 CECs *detected in the invasive species analyzed (P. clarkii, C. fluminea, L. gibbosus) and the native species (A. cygnea) in at least one exposure site. The detected compounds were classified as PhACs (black), pesticides (green), PFAS (grey), OPFRs (orange) and other compounds of industrial use (purple). Overlapping areas indicate compounds that have been detected in more than one species. O-dmvlf: O-desmethylvenlafaxine. Terb: Terbuthylazine.*

770 ng g^{-1} and 21–180 ng g^{-1}, respectively [34].

Pesticides exhibited higher concentrations in surface water and thus, a higher percentage of contribution to the total sum at the sampling sites located at the lake (both north and south), and in the Comú (1343, 1486, and 1425 ng L^{-1}, respectively). The elevated pesticide concentrations at the lake could be explained by the confluence of water from irrigation channels, which carry pesticides applied in the surrounding agricultural areas [17]. Furthermore, the high concentrations obtained were mainly due to the presence of the fungicide azoxystrobin, which exceeded 1300 ng L^{-1} at the three mentioned sites, being the main contributor to the total concentrations. This fungicide, widely used in wheat crops to prevent and treat blast infestations, has previously been detected in similar concentrations in water samples from ANP, as it is used in the region's rice crops at the end of the summer period [19].

However, the pesticides obtained the lowest detection frequency among the species studied. No compound was detected simultaneously in all four species. For *P. clarkii* and *A. cygnea*, the compound with the highest median concentration was terbuthylazine, with 34.8 ng g^{-1} and 18.9 ng g^{-1} respectively, at Camptes. For *C. fluminea*, the compound with the highest concentration was the carbofuran-3-hydroxy metabolite (28.6 ng g^{-1}) in C. Font Nova, while for *L. gibbosus* it was atrazine-desethyl (10.6 ng g^{-1}) in Lake North.

Despite the restrictions imposed on their use [35,36], the presence of triazines continues to be reported in aquatic ecosystems because they are historically among the most widely used and persistent pesticides. Their presence in this ecosystem could be explained, in addition to the residues existing from previous use, by the contributions made by the Turia River to the ANP, since these types of compounds have been previously reported in this river [37]. As far as we know, no studies have reported the presence of the specific pesticides included in this research in *P. clarkii*, *L. gibbosus*, or *A. cygnea*. Therefore, we could not conduct a comparative analysis of the concentrations presented here.

3.2. Patterns and species differences in the accumulation of CECs

A Kruskal-Wallis test was performed to assess statistical differences in CECs concentrations across species by compounds category. As shown in Fig. 4, no statistically significant differences were observed for OPFRs and PFAS groups, indicating similar concentrations across species. This suggests no particular susceptibility to accumulation in any single species, and that the observed variations are probably due to fluctuations in water concentrations rather than in the species' ability to accumulate.

When comparing the native clam *A. cygnea* with the invasive species, it was observed that *A. cygnea* accumulated higher concentrations of a greater number of compounds compared to *P. clarkii* and *L. gibbosus,* except in the case of pesticide metabolites and stimulants, to which *P. clarkii* may provide more relevant

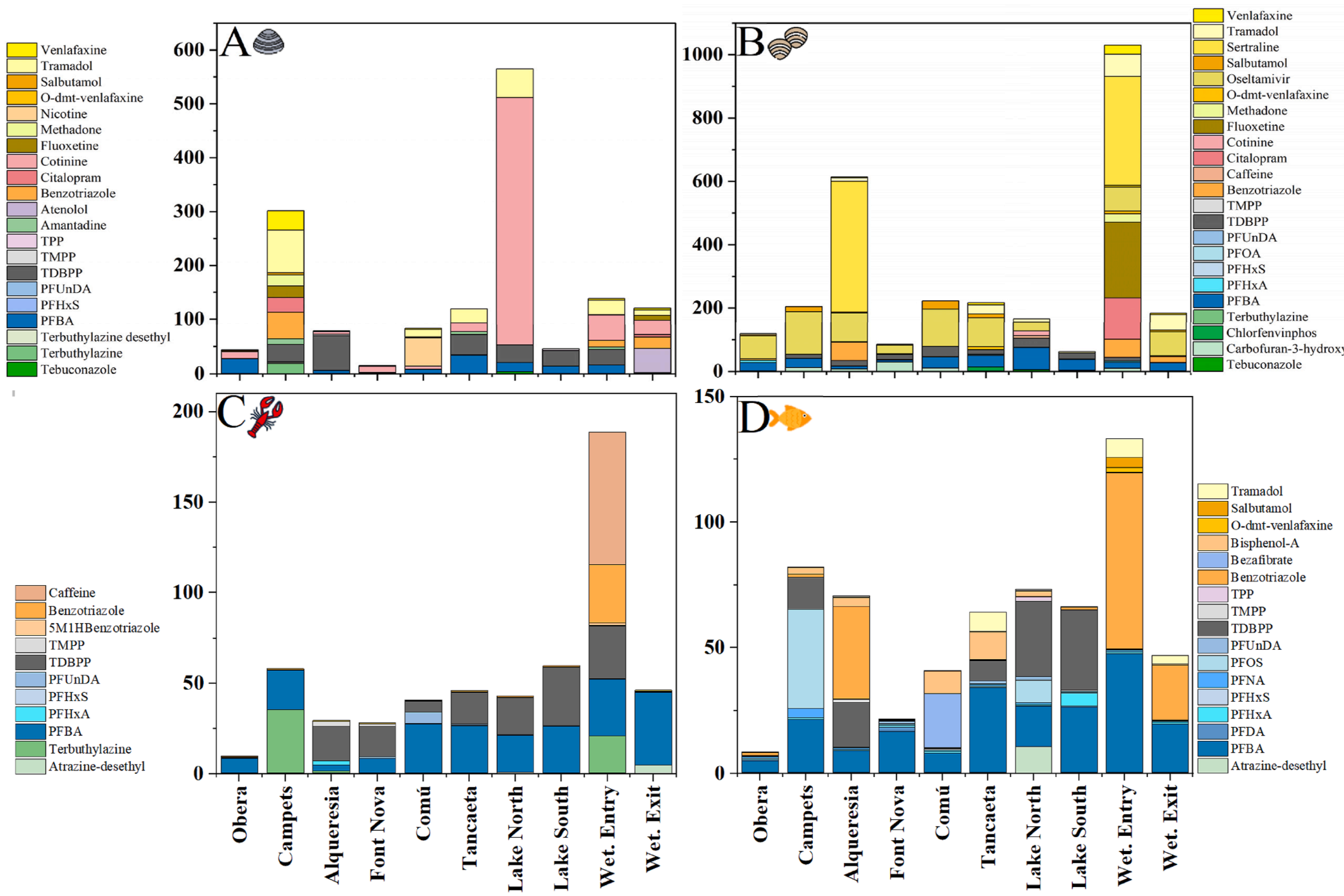

Fig. 3. *Distribution of CECs by species and sampling site.* ***A:*** *A. cygenea;* ***B:*** *C. fluminea;* ***C:*** *P. clarkii;* ***D:*** *L. gibbossus. The figure shows median concentrations in ng g^{-1}(w.w) calculated for each compound on the y-axis, and the exposure sites organized by channels, lake, and artificial wetland on the x-axis. The legend of each box shows the compounds detected in each of the species.*

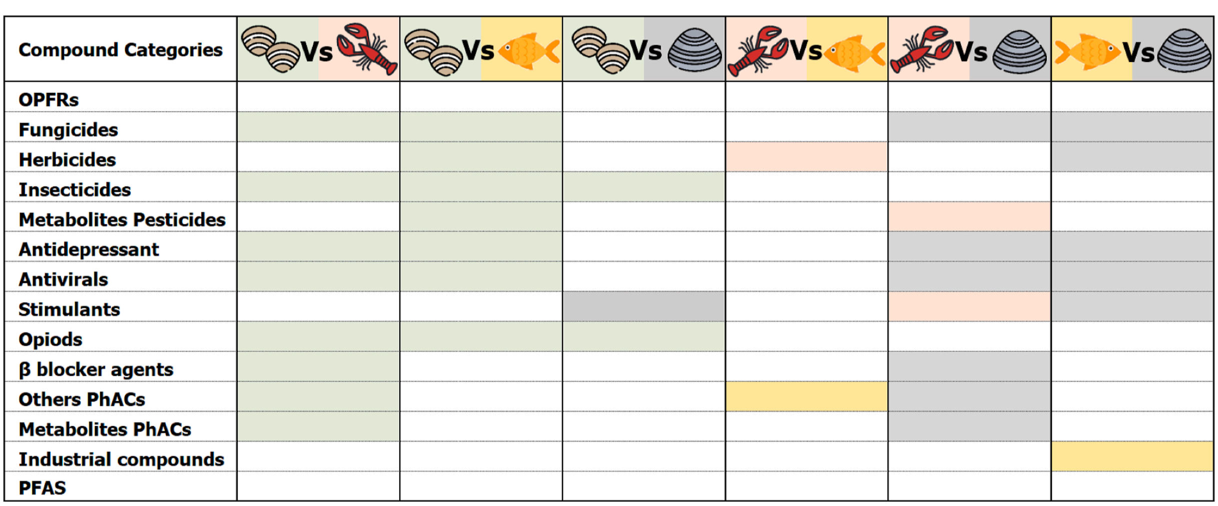

Fig. 4. Statistically significant differences in CECs accumulation between the species analyzed according to the Kruskall-Wallis test. Blank boxes indicate no significant differences between species pairs, while colored boxes indicate a significant difference (p-value <0.05). Each color represents the species with the highest concentration for a specific category of compounds: green for C. fluminea, red for P. clarkii, yellow for L. gibbosus, and grey for A. cygnea.

information, and for industrial compounds, to which *L. gibbosus* showed greater accumulation. However, when compared to the invasive species *C. fluminea*, the significant differences were smaller, likely because both species belong to the same taxonomic group and share similar characteristics and accumulation patterns. These differences in accumulation could be related to the water filtration capacity of *C. fluminea*, making it more efficient at absorbing contaminants from the aquatic environment. It has been estimated that *C. fluminea* can reach a filtration rate of 3000 mL h^{-1} per individual, which is ten times higher than that of similarly sized native species, whose rates range between 150 and 300 mL h^{-1} per individual [38].

Differences in accumulation patterns between the species analyzed can be attributed to factors such as diet and morphological and physiological traits. Studies have shown that body size, surface area, and volume can influence the bioaccumulation of organic contaminants, with bioaccumulation being inversely proportional to the volume or weight of different species [39]. In this study, *A. cygnea* and *C. fluminea* are the smallest species, with average sizes of 40–45 mm and 25 mm, respectively. In contrast, the crayfish *P. clarkii* has an average size of 18 cm, while *L. gibbosus* measures around 8 cm. The smaller size and larger surface-area-to-volume ratio of filtering tissue of *C. fluminea* could have contributed to higher concentrations for most of the compound categories.

The high concentrations in clams, particularly in *C. fluminea*, could also be attributed to species-specific biotransformation processes. It has been shown that bivalves have less efficient enzymatic systems for the biotransformation of chemical contaminants compared to fish. Bivalves can accumulate contaminants more readily, but they metabolize and eliminate them more slowly than vertebrates [40]. This slower rate of metabolism could contribute to the greater retention of contaminants in their tissues.

The nature of species' body surfaces can affect the diffusion rates of contaminants through direct water contact [41]. Among the analyzed species, there are differences in how their body surfaces interact with the aquatic environment. For example, *P. clarkii* possesses a semi-permeable chitin exoskeleton, which acts as a protective barrier and may reduce the absorption of less lipophilic contaminants [42]. In contrast, **fish**, like *L. gibbosus*, particularly juveniles and larvae, tend to have higher dermal absorption rates, primarily due to the thinner skin and lack of fully developed scales, which increases as they grow. This allows for more direct contaminant absorption through the skin during the early stages of life, but it decreases as they mature and their protective layers develop [43,44].

On the other hand, clams such as *A. cygnea* and *C. fluminea* are active filter feeders, a behavior that increases the volume of water, and thus, contaminants in contact with their internal tissues. This elevated exposure can enhance the accumulation of certain compounds, particularly weak bases. In these organisms, it has been suggested that the bioaccumulation of weak bases may be driven not only by lipid partitioning but also by electrostatic interactions, where the positively charged form of the compound is attracted to the negatively charged cell membranes [45]. This feature could explain the significant differences for antidepressants such as venlafaxine and its metabolite o-desmethylvenlafaxine, sertraline, fluoxetine and, citalopram; the b-blocker tramadol; the antiviral oseltamivir; the opioid methadone and compounds such as salbutamol and cotinine, which were detected mainly in *C. fluminea,* and with higher concentrations.

3.3. Bioaccumulation factors (BAFs) and the influence of exposure sites

BAFs were calculated for 22 compounds with concentrations > LOQ that were detected simultaneously in at least one of the species and at least one of the exposure sites (Fig. 5). A compound was considered bioaccumulative if its BAF was equal to or greater than 1000 [8]. The site with the highest BAFs was Wet Entry, where BAF values greater than 1000 were recorded for twelve cases: PFBA and cotinine in *A. cygnea*; PFBA, citalopram, fluoxetine, methadone, and salbutamol in *C. fluminea*; terbuthylazine, PFBA, and caffeine in *P. clarkii*; and PFBA and o-desmethylvenlafaxine in *L. gibbosus*. In second place, the exposure site Camptes recorded BAFs>1000 for seven cases.

The high BAF values observed at Wet. Entry can be attributed to its role as the inlet of the Milia artificial wetland, a former rice field adjacent to ANP that has been converted into a constructed wetland or green filter for water treatment before it reaches the lake [46]. This artificial wetland receives treated water from the Albufera Sur WWTP located 10 km away [18]. On the other hand, there is clear evidence of a reduction in the concentrations of compounds in the effluent treated by the artificial wetland, as reflected by the fact that no BAF values above 1000 were observed in the species exposed at Wet Exit. This suggests that the wetland's treatment process effectively reduces contaminants' bioavailability, lowering their accumulation in aquatic organisms downstream of the constructed wetland.

The highest BAFs were calculated for fluoxetine in *C. fluminea* (52795); PFBA in *L. gibbosus* (from 5842 to 29113), *P. clarkii* (from 2174 to 19046), and *C. fluminea* (from 5646 to 11796) and; for cotinine in *A. cygnea* (from 153 to 15718). The species with the highest number of BAF values exceeding 1000 was *A. cygnea*, with 14 cases (each case represents the BAF value of a compound at a specific exposure site). Notably, *A. cygnea* showed elevated BAF values for compounds such as cotinine, and tramadol (from 16 to 2245), and for terbuthylazine desethyl, PFBA, amantadine, o-desmethylvenlafaxine, and venlafaxine.

Following this*, C. fluminea* exhibited BAF>1000 in nine cases, particularly for compounds like PFBA, citalopram, fluoxetine, methadone, and salbutamol. *P. clarkii* had seven cases, primarily for terbuthylazine, PFBA, PFUnDA, and caffeine. Lastly, *L. gibbosus* showed five cases, specifically for atrazine-desethyl, PFBA, and salbutamol. This variation in bioaccumulation could be attributed to species-specific physiological traits and their interaction with environmental factors at the various exposure sites.

The bioaccumulation capacity of antidepressants in *C. fluminea* has been previously reported [28], who exposed this species downstream of a WWTP in Pecan Creek Lake, USA. After 42 days of exposure, they reported BAF values ranging from 454 to 702 for fluoxetine and from 3361 to 6485 for sertraline. While the BAF for fluoxetine observed in this study is lower than the values we report, both studies highlight *C. fluminea's* strong bioaccumulation potential for this class of compounds. Unfortunately, we were unable to calculate a BAF for sertraline, as the compound was not quantified in water samples; however, its concentration in the tissue was among the highest recorded (344–413 ng g^{-1}).

The elevated BAF values observed in clams, particularly in *A. cygnea*, are primarily attributed to their role as filter-feeders. Bivalves are capable of filtering large volumes of water, processing microalgae, bacteria, and organic matter while also capturing particles from both, the water column and the sediments. This filtering capacity allows them to ingest contaminants present in the environment, especially from sediments, which is one of the main pathways through which pollutants accumulate in their tissues [45,47]. These characteristics grant bivalves a higher absorption and accumulation potential compared to the other species analyzed in this study.

Regarding the differences between the two-clam species, *A. cygnea* appears to have a higher bioaccumulation efficiency for certain contaminants, likely due to its physiological traits, including protein-binding capacity and lipid content. *A. cygnea* has a lipid percentage of 2.2 %, compared to *C. fluminea*, which has only 0.7 % [22]. This difference in lipid content could explain why contaminant concentrations tend to be higher in *A. cygnea*, as compounds with a higher affinity for lipids or proteins may

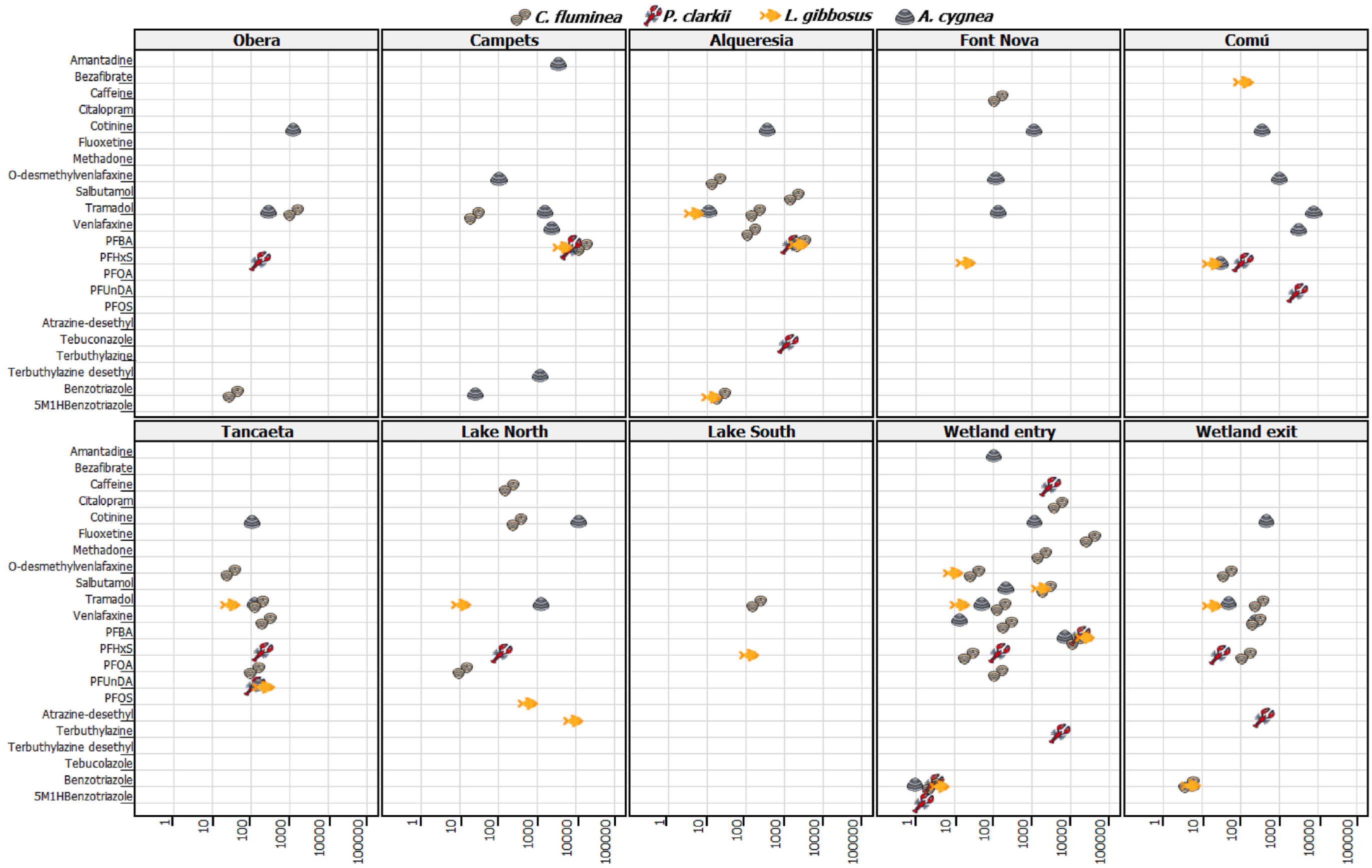

Fig. 5. BAFs per species and exposure site for CECs with mean concentrations > LOQ.

accumulate more efficiently in species with higher fat reserves.

Given the lipid content of *P. clarkii* (3.0 %) [22], higher BAFs, especially for nonpolar compounds, would be expected. However, its bioaccumulation capacity may have been limited by exposure conditions, where access to detritus, macrophytes, mollusks, insects, annelids, tadpoles, and small fish its typical diet was restricted due to confinement to water and pelagic organisms only. Additionally, compared to clams, it filters smaller volumes of water and its exoskeleton is impermeable [13,48].

Another factor that can influence the bioaccumulation of CECs in aquatic organisms is the physicochemical nature of the compounds, particularly their hydrophobicity, commonly expressed as the octanol–water partition coefficient (log K_{ow}). Generally, compounds with higher log K_{ow} values exhibit a stronger affinity for lipid-rich tissues, which tends to enhance their bioaccumulation potential and reduce their solubility and mobility in water. To evaluate the relationship between hydrophobicity and bioaccumulation, we compared the experimentally derived BAFs with the corresponding log K_{ow} values for each compound across the four studied species (Fig. S3). Although a general trend of increased BAF with increasing log K_{ow} could be expected, our results did not show a consistent or strong correlation across all species. For example, in *A. cygnea* and *C. fluminea*, several compounds with moderate log K_{ow} values (2–4) exhibited relatively high log BAFs, while other compounds with higher log K_{ow} (>5) did not necessarily result in higher bioaccumulation. Similarly, in *P. clarkii* and *L. gibbosus*, the variability in BAF across the log K_{ow} spectrum suggests that additional factors are influencing compound uptake and retention. These observations indicate that while lipophilicity is an important determinant, it is not the sole driver of bioaccumulation. Other mechanisms such as active uptake, biotransformation, and species-specific physiological or ecological traits (e.g., feeding strategy, metabolism or lipid content) likely contribute to the observed variability. Furthermore, the environmental behavior of CECs, including their persistence, binding to particulate matter, or transformation into metabolites, could also affect their bioavailability and subsequent bioaccumulation. Therefore, although log K_{ow} provides useful insight into a compound's potential for bioaccumulation, it should be interpreted in the context of a broader set of biological and environmental factors to better understand species-specific accumulation patterns under field conditions.

In the case of *A. cygnea*, the accumulation of cotinine, a polar compound with a low log K_{ow} (−0.23) and generally not expected to bioaccumulate significantly was particularly notable. Despite its hydrophilic nature, cotinine was detected in relatively high concentrations in this species, suggesting that factors beyond compound lipophilicity may influence its bioaccumulation. This may be attributed to the physiology of *A. cygnea* as a filter-feeding organism, capable of retaining a wide spectrum of contaminants through the ingestion of suspended particles and sediments. Such feeding behavior facilitates the uptake of substances of different physicochemical properties. The presence of cotinine could indicate a limited capacity of this species to biotransform and/or excrete this metabolite. Although cotinine is widely recognized as the primary metabolite of nicotine and has been proposed as a marker of tobacco consumption in wastewater [49], little is known about its fate in aquatic organisms. Its presence in environmental matrices has been linked to contamination from cigarette butts and nicotine leachates, which may act as vectors for

Species	Compounds	PNEC µg Kg^{-1} w.w.	Obera	Campets	Alqueresía	Font Nova	Comú	Tancaeta	Lake North	Lake South	Wet. Entry	Wet. Exit
A. cygnea	Tebuconazole	2.33							1.68			
	Terbuthylazine	0.32										
	Terbuthylazine desethyl	0.04										
	PFBA	46.3										
	PFHxS	0.22										
	PFUnDA	55.9										
	TDBPP	16.9		1.87	3.74			2.22	1.88	1.67	1.67	
	TMPP	6.72										
	TPP	3.27										
	Amantadine	376										
	Atenolol	3.94										
	Benzotriazole	27.2		1.80								
	Citalopram	411										
	Cotinine	6.90	1.74			1.57		2.28			6.73	3.69
	Fluoxetine	9.45		2.23								
	Methadone	31.2										
	Nicotine	11.7					4.44					
	O-dmt-venlafaxine	9.86										
	Salbutamol	1205										
	Tramadol	129										
	Venlafaxine	22.0		1.65								
Hazard index			2.34	125	4.99	1.64	5.88	5.46	70.8	2.93	9.58	17.0
C. fluminea	Tebuconazole	2.33							1.97			
	Carbofuran-3-hydroxy	12.5				2.28						
	Chlorfenvinphos	6.38						1.98				
	Terbuthylazine	0.32								5.16		
	PFBA	46.3							1.51			
	PFHxA	1725										
	PFHxS	0.22										
	PFOA	0.10						2.90			6.80	
	PFUnDA	55.9										
	TDBPP	16.9			1.02		1.90		1.69	1.13		
	TMPP	6.72										
	Benzotriazole	27.2			2.12						2.09	
	Caffeine	0.48				2.88						
	Citalopram	411										
	Cotinine	6.90							2.12			
	Fluoxetine	9.45										
	Methadone	31.2										
	O-dmt-venlafaxine	9,86										
	Oseltamivir	6,78				4,12			4,08			
	Salbutamol	1205										
	Sertraline	0,33										
	Tramadol	129										
	Venlafaxine	22,0									1,31	
Hazard index			11,5	22,1	1269	10,7	20,8	22,3	28,1	7,08	1095	13,7
P. clarkii	Atrazine-desethyl	0,44							2,34			
	Terbuthylazine	0,32			3,28							
	PFBA	46,3										
	PFHxA	1725										
	PFHxS	0,22										
	PFUnDA	55,9										
	TDBPP	16,9			1,12			1,05	1,20	1,91	1,74	
	TMPP	6,72										
	5M1HBenzotriazole	229										
	Benzotriazole	27,2									1,18	
	Caffeine	0,48										
Hazard index			0,70	109	4,87	1,61	1,47	2,36	3,99	2,47	220	12,5
L. gibbosus	Atrazine-desethyl	1,76							6,03			
	PFBA	185										
	PFDA	8,15										
	PFHxA	6901										
	PFHxS	0,88										
	PFNA	165,0										
	PFOS	51,7										
	PFUnDA	223										
	TDBPP	67,7										
	TMPP	26,9										
	TPP	13,1										
	Benzotriazole	109										
	Bezafibrate	13,6				1,58						
	Bisphenol-A	13,1										
	O-dmt-venlafaxine	39,5										
	Salbutamol	4822										
	Tramadol	515										
Hazard index				1,30	0,97	0,32	2,34	1,16	7,03	0,79	0,97	0,34

0.1-1	Medium risk		
<0.1	Minimal risk	>1 High risk	>1000
			100-1000
			10-100
			1-10

Fig. 6. Calculated risk quotient for each single compound at each site, and calculated hazard index for each species in each sampling site.

a range of toxicants including nicotine [50]. Therefore, further research on the metabolic pathways and detoxification mechanisms of cotinine in *A. cygnea* is necessary to better understand its persistence and potential impacts.

C. fluminea and *P. clarkii*, on the other hand, showed a greater tendency to bioaccumulate compounds with a higher log K_{ow} (>4) such as PFHxS, which is consistent with the theory that more lipophilic compounds tend to accumulate more readily in fat-rich tissues. In particular, these results are consistent with the idea that apolar compounds show a higher affinity for organisms with higher lipid content, as is the case of *P. clarkii*, which has a lipid percentage of 3 %, the highest concerning the other study species. Finally, in *L. gibbosus*, although high BAFs were also observed for some compounds with intermediate log K_{ow} values, such as PFUnDA and PFBA, the overall accumulation seems to be less influenced by the compounds' lipophilicity compared to other species, suggesting that other factors, such as metabolism and the specific physiology of this species, also play a relevant role.

Fig. S4 shows the average BAFs for each compound, calculated across the different exposure sites. Distinct accumulation patterns were observed depending on the compound and the species analyzed. *C. fluminea* exhibited a marked tendency to bioaccumulate pharmaceuticals such as citalopram, fluoxetine, and methadone, with fluoxetine reaching BAF values above 10000. In contrast, *A. cygnea* showed the highest accumulation levels for compounds like tramadol, amantadine, atenolol, nicotine, and its metabolite cotinine. These differences highlight that bioaccumulation capacity varies among species, potentially due to specific physiological or ecological traits. Moreover, the large standard deviations observed for some compounds suggest that local exposure conditions may also influence accumulation levels as detailed above. In addition, it is relevant to note that the calculated BAFs are based on water concentrations measured at specific times during the experimental development, which introduces greater uncertainty when trying to obtain accurate BAF values.

3.4. Ecological risk assessment

In this section, to evaluate the risk of each detected compound, risk quotients (RQ) were calculated (Fig. 6). Henceforth, to facilitate the understanding of the discussion, the combination of species, compound, and exposure site will be referred to as a "case". The highest RQs were observed for sertraline in *C. fluminea* at the Alqueresia and Wet. Entry sites, with values of 1251 and 1043, respectively. *C. fluminea* presented medium RQ values in 36 cases and high RQs in 29 cases. Notably, median concentrations of oseltamivir exceeded the PNEC at all exposure sites except Lake South, with RQs ranging between 4.1 and 19.8. Measured concentrations in *A. cygnea* exceeded the PNEC for 17 compounds, reaching medium risk in 26 cases and high risk in 20 cases. The highest RQs for this species were calculated for cotinine (66.5) at Lake North, terbuthylazine (59.2) and its metabolite terbuthylazine desethyl (56.2) at Camptes, and atenolol (11.3) at Wet. Exit.

The effects of oseltamivir have been demonstrated in *Mytilus galloprovincialis* (mussels) and *Ruditapes philippinarum* (clams). In mussels, exposure to oseltamivir caused a significant increase in glutathione reductase (GR) activity in the gills, with no marked antioxidant response observed in the digestive gland. In addition, lipid peroxidation (LPO) levels were significantly reduced in mussels, indicating possible protective effects against oxidative damage. No response in gill antioxidant defenses was evident in clams, but LPO levels were significantly elevated, suggesting increased oxidative stress [51].

P. clarkii exhibited **RQs** greater than 1.0 in 12 cases, and between 0.1 and 1.0 in 19 cases. The highest RQs for this species were recorded for **caffeine** (152) at Wet. Entry, **terbuthylazine** (109) at Camptes, and **atrazine-desethyl** at Wet. Exit. Previous studies have shown that crayfish exposed to atrazine displayed impaired movement, which indicates potential neurological effects. Additionally, exposure to terbuthylazine has been associated with histological damage, oxidative stress, and alterations in antioxidant biomarkers [52].

The species with the fewest cases where RQ values exceeded 1.0 was *L. gibbosus*, which surpassed this threshold in only two cases: for atrazine desethyl (6.0) at Lake North and bezafibrate (1.6) at Comú. In the group of PFAS, RQs were reported below 7.0, with the highest values being for PFOA in *C. fluminea* (6.8) and PFBA in *A. cygnea* (1.5). Regarding OPFRs, RQs remained below 4.0, with the highest values reported for TDBPP in *A. cygnea* (from 1.7 to 2.2).

According to the hazard index (HI), which evaluates the level of exposure to a mixture of substances per site for each species, *C. fuminea* presented a high risk in most of the exposure sites, with HI values ranging from 11.5 in Obera to 1269 in Alqueresia. It was observed that the risk of mixing substances at each exposure site varied by species, with *L. gibbosus* having the lowest HI. However, at most sites, HI values posed a high risk, indicating strong toxic pressure exerted by the CECs mixture for both native and invasive species.

4. Conclusions

This study identified the presence of 35 CECs, primarily PhACs and perfluorinated carboxylic acids (PFAS), in native and invasive freshwater species of the Albufera Natural Park. The highest concentrations and BAFs in invasive species were found at the inlet of the artificial wetland. Bivalves (*A. cygnea* and *C. fluminea*) accumulated a broader range of CECs than other species, with fluoxetine and cotinine showing the highest BAFs. These results confirm the suitability of *C. fluminea* as an effective biomonitor for this aquatic ecosystem, given its ability to accumulate a wide variety of compound families. Minimal differences in accumulation patterns were observed between *C. fluminea* and *A. cygnea*, suggesting that the invasive clam can reflect contaminant presence with a reliability comparable to that of native species.

Risk assessment results indicated that PhACs, particularly the antidepressant sertraline and the antiviral oseltamivir, pose the greatest potential risk to aquatic organisms in the park, followed by certain herbicides and their transformation products. *C. fluminea* was the species that most frequently exceeded reference values, showing the highest internal contaminant concentrations. This could indicate a greater resistance to chemical exposure, although compound-specific toxicological studies are needed to verify this hypothesis.

One of the key contributions of this work is the demonstration of the value of using invasive species for environmental biomonitoring. Their deployment provided critical insights into localized chemical exposure and identified sertraline, fluoxetine, terbuthylazine, caffeine, and oseltamivir as priority CECs. Moreover, this approach reduces the impact on native species and offers a dual benefit, supporting conservation goals by minimizing competition from invasive populations, while improving chemical risk assessment in vulnerable ecosystems.

CRediT authorship contribution statement

Diana P. Manjarrés-López: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation. **Claudia Martínez-Megías:** Methodology, Data curation. **Dyana Vitale:** Methodology. **Yolanda Picó:** Writing – review &

editing, Funding acquisition, Data curation. **Andreu Rico:** Writing – review & editing, Funding acquisition, Data curation, Conceptualization. **Sandra Pérez:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Investigation, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.emcon.2025.100533.

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