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Moisès Sánchez-Fortún [a,b,*], Josep Lluís Carrasco [c], Sergi Díez [d], David Amouroux [e], Emmanuel Tessier [e], Sophie López-Carmona [a,f], Carola Sanpera [a,b]

[a] Department of Evolutionary Biology, Ecology and Environmental Sciences, University of Barcelona, Barcelona, Spain
[b] Institut de Recerca de la Biodiversitat (IRBio), University of Barcelona, Barcelona, Spain
[c] Biostatistics, Department of Basic Clinical Practice, University of Barcelona, Barcelona, Spain
[d] Environmental Chemistry Department, Institute of Environmental Assessment and Water Research, IDAEA-CSIC, E-08034, Barcelona, Spain
[e] Université de Pau et des Pays de L'Adour, E2S UPPA, CNRS, IPREM, Institut des Sciences Analytiques et de Physico-chimie pour l'Environnement et les Matériaux, Pau, France
[f] UFR Sciences et Techniques, Université de Nantes, Nantes, France

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ABSTRACT

During the last few decades, inputs of mercury (Hg) to the environment from anthropogenic sources have increased. The Ebro Delta is an important area of rice production in the Iberian Peninsula. Given the industrial activity and its legacy pollution along the Ebro river, residues containing Hg have been transported throughout the Ebro Delta ecosystems. Rice paddies are regarded as propitious environments for Hg methylation and its subsequent incorporation to plants and rice paddies' food webs. We have analyzed how Hg dynamics change throughout the rice cultivation season in different compartments from the paddies' ecosystems: soil, water, rice plants and fauna. Furthermore, we assessed the effect of different agricultural practices (ecological vs. conventional) associated to various flooding patterns (wet vs. mild alternating wet and dry) to the Hg levels in rice fields. Finally, we have estimated the proportion of methylmercury (MeHg) to total mercury in a subset of samples, as MeHg is the most bioaccumulable toxic form for humans and wildlife. Overall, we observed varying degrees of mercury concentration over the rice cultivation season in the different compartments. We found that different agricultural practices and flooding patterns did not influence the THg levels observed in water, soil or plants. However, Hg concentrations in fauna samples seemed to be affected by hydroperiod and we also observed evidence of Hg biomagnification along the rice fields' aquatic food webs.

1. Introduction

Mercury (Hg) is a globally distributed pollutant that has been rising its levels in the environment due to human activities (e.g., artisanal gold mining, industrial waste management, etc.) during the last decades (UNEP, 2019). This metal can exert serious negative consequences on the health of both wildlife and humans. Among others, reproductive, neurological, physiological, and teratogenic impairments have been observed on wildlife and humans exposed to Hg (Clarkson and Magos, 2006; Díez, 2009; Evers, 2018).

Mercury is present in all environmental compartments (air, soil, water and biota), and speciation processes during its biogeochemical cycle are highly dependent on the environment physicochemical factors (Ullrich et al., 2001; Driscoll et al., 2013). In the aquatic environment, inorganic mercury (Hg^{2+}) can be methylated by microorganisms, mainly sulfate-reducing bacteria (SRB), iron-reducing bacteria (IRB; Podar et al., 2015), and methanogenic archaea (Lázaro et al., 2013), or abiotic processes (Celo et al., 2006) to become methylmercury (MeHg). MeHg is a bioavailable form of mercury and can be bioaccumulated into organisms and biomagnified along food webs (Morel et al., 1998; Gray, 2002; Driscoll et al., 2013; Lavoie et al., 2013; Córdoba-Tovar et al., 2022).

Wetland environments are often linked to high MeHg production rate (Zillioux et al., 1993; Marvin-DiPasquale et al., 2014). Progressively

* Corresponding author. Department of Evolutionary Biology, Ecology and Environmental Sciences, University of Barcelona, Barcelona, Spain and Institut de Recerca de la Biodiversitat (IRBio), University of Barcelona, Barcelona, Spain.
E-mail addresses: m.sanchez-fortun@ub.edu (M. Sánchez-Fortún), jlcarrasco@ub.edu (J.L. Carrasco), sergi.diez@idaea.csic.es (S. Díez), david.amouroux@univ-pau.fr (D. Amouroux), emmanuel.tessier@univ-pau.fr (E. Tessier), sophielopezcarmona@gmail.com (S. López-Carmona), csanpera@ub.edu (C. Sanpera).

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with the worldwide population growth, natural wetlands tend to disappear and are replaced by agricultural farming site such as rice paddies (Elphick, 2000; Verhoeven and Setter, 2010; Davidson, 2014). Although fish and shellfish consumption is considered the main source of mercury for humans (Mergler et al., 2007; McKelvey and Oken, 2012; UNEP, 2019), some studies suggest that rice consumption might be another route for mercury exposure that should not be neglected (e.g., Feng et al., 2008; Li et al., 2010; Meng et al., 2011; Hong et al., 2016; Zhao et al., 2019; Liu et al., 2019). Indeed, flooded rice paddies are considered as hotspots for mercury methylation, as anaerobic conditions in rice field soils promote Hg methylation by microorganisms (Rothenberg and Feng, 2012). This MeHg formed in the rice paddies can be incorporated into the rice plants (Meng et al., 2011; Wang et al., 2018). Furthermore, MeHg in rice paddies might also impact animals inhabiting these environments (Ackerman and Eagles-Smith, 2010; Ackerman et al., 2010; Abeysinghe et al., 2017; Du et al., 2021; Sánchez-Fortún et al., 2021).

Among the factors influencing the bioavailability of mercury in rice paddies, the effects of agricultural techniques used in rice farming (e.g., continuous flooding, alternating wetting-drying periods, use of pesticides) has been evaluated in several studies (e.g., Rothenberg and Feng, 2012; Rothenberg et al., 2011, 2014; Marvin-DiPasquale et al., 2014; Tang et al., 2018; Tanner et al., 2018). Among them, the alternating wetting and drying (AWD) technique, characterized by a drainage just after seeding followed by periods of intermittent wetting and drying of the field (Bouman and Tuong, 2001; Linquist et al., 2015), has been associated with higher MeHg production in agricultural wetlands (Marvin-DiPasquale et al., 2014; Rothenberg et al., 2014). However, other experimental studies (e.g., Rothenberg et al., 2016; Tanner et al., 2018) have observed reductions in MeHg levels in rice plants growth under AWD conditions compared to continuously flooded conditions. This highlights the multifactorial effects of these agricultural techniques on Hg availability and methylation within rice fields (Rothenberg et al., 2016; Tanner et al., 2018; Zhao et al., 2016a; Tang et al., 2020).

Over the last century, the presence of a chlor-alkali plant at the locality of Flix (Tarragona, NE Spain), ca. 100 km upstream the Ebro river mouth, has impacted the ecosystems along the river and its delta (Navarro et al., 2009; Suárez-Serrano et al., 2010; Carrasco et al., 2011a, 2011b; Cotín et al., 2012; Palanques et al., 2020; Sánchez-Fortún et al., 2020). During the active period of the plant, around 350 000 tons of toxic waste, containing mainly organochlorine compounds and metals (including Hg), were dumped into the riverbed (Grimalt et al., 2003; ACA, 2013). Despite plant operations producing chlorine using mercury ceased in 2017, legacy mercury pollution persists and has been impacting downstream Ebro River basin environments due to the transport of pollutants along the river (Palanques et al., 2020; Sánchez-Fortún et al., 2020).

The Ebro Delta is comprised by 30 000 ha of wetland environments, with almost 75 % of its area occupied by rice fields, 20 % of its area protected as a natural reserve, and 5 % being urbanized (Mañosa et al., 2001). Water from the Ebro river is used to flood continuously or alternately the rice paddies in the Ebro Delta area (Terrado et al., 2007). Thus, the potential absorption of contaminants from the water-soil system by rice plants in the Ebro delta can lead to higher toxicological risk for wildlife and humans through biomagnification. This is of highest concern in the area, as the Ebro Delta is one of the most important areas in the Iberian Peninsula both as rice farming site and natural reserve (Mañosa et al., 2001).

In this context, the study of mercury dynamics in rice fields provides an estimate of mercury budgets in different compartments of the agricultural ecosystems and their interconnectivity. The main purpose of this study is to describe the dynamics of Hg in the rice paddies ecosystems from the Ebro Delta along the rice cultivation season, including the following compartments: water, soil, rice plants and food webs within rice fields. Additionally, the effect of different agronomic practices used by rice farmers in the Ebro Delta was evaluated regarding the mercury observed in the different environmental compartments considered. Furthermore, mercury speciation analyses to determine the proportion of MeHg were performed for a subset of samples to evaluate the temporal dynamics of MeHg, as a proxy of the bioavailable fraction of mercury in the different compartments of the rice paddies environments.

2. Materials and methods

2.1. Field sampling design

Field work was conducted in the Ebro Delta area (NE Spain, 40°33′ N, 00°39’ E). During the rice cultivation season of 2017, eight rice fields from the area were randomly selected (Fig. S1), which were cultivated by farmers using different agronomical practices, as follows: (i) 4 rice fields using a conventional wet (CW) approach, that is, flooded throughout the cultivation season and using fertilizers and pesticides; (ii) 2 rice fields using an ecological wet (EW) approach, consisting in flooded fields throughout the cultivation season without any usage of fertilizers or pesticides; and (iii) 2 rice fields managed using an ecological dry (ED) approach, where pesticides or fertilizers were not used and the fields were flooded only during half of the rice growing period using a mild alternating wet and dry (AWD) method.

In each of the eight rice fields, we selected 4 sampling points, one in each side of the field, to account for the variability within the field in our measurements. At each sampling point total mercury concentrations ([THg] ng/g dw) were measured for water, soil, rice plants, and fauna inhabiting the rice field (as described below) and the following physicochemical parameters were measured for the overlying water: pH, salinity (%), conductivity (mS), temperature (°C) and oxygen concentration (mg/L). Furthermore, for animal samples, carbon and nitrogen stable isotopes signatures were measured.

This sampling design was performed three times throughout the rice growing season: (1) at the end of June/beginning of July, two months after the rice was seeded; (2) in August, three months after the rice was seeded; and (3) in September, at the end of the rice growing season.

Finally, for a subset of rice plants, fauna samples and soil samples corresponding to the first, second and third sampling periods (i.e., June/July, August and September, respectively) from the ecological wet (EW) rice fields, speciation analyses were performed to measure the concentration of MeHg and inorganic mercury (iHg) in the samples.

2.2. Sample collection and laboratory analyses

2.2.1. Water

For the determination of the labile fraction of Hg in the overlying water, we used the diffusive gradient in thin films (DGT) technique (Turull et al., 2017a; Marrugo-Madrid et al., 2021). DGTs were deployed for 7 days in each sampling point for the first (June/July) and third (September) sampling periods. Upon collection, DGTs were stored at -4 °C until analysis. Total mercury (THg) analysis in resin gels were made using a Direct Mercury Analyzer (DMA-80, Milestone Inc.) according to the EPA method 7473 (USEPA, 1998), following Marrugo-Madrid et al. (2022).

Concentrations in the labile fraction of Hg in water were evaluated for a total of N = 56 DGTs, which included 32 samples for the CW fields, 16 samples for the EW fields and 8 samples for the ED fields. In the ED fields, we could not deploy the 8 DGTs corresponding to the third sampling period (September) as the field was already dried out prior to harvesting (Table 1).

2.2.2. Soil samples

Soil samples were obtained by extracting a ~5 cm depth core of sediments from non-rizosphere areas at each sampling point for each sampling period (N = 96 soil cores). Samples were stored at −20 °C until analysis. Before the determination of total mercury concentration, samples were homogenized and were oven-dried at 50 °C until constant

Table 1
Sample size (N) and summary statistics (mean ± SE) for THg concentration (ng/g dw or ng/L) measurements for each of the sample types (soil, water (DGTs) and rice plant parts), agricultural management type, and sampling period obtained during the 2017 rice cultivation season.

Sample type	Sampling period	Conventional Wet (CW)		Ecological Wet (EW)		Ecological Dry (ED)	
		N	THg (ng/g dw or ng/L)	N	THg (ng/g dw or ng/L)	N	THg (ng/g dw or ng/L)
Soil	1	16	224.9 ± 41.0	8	157.9 ± 17.6	8	204.4 ± 47.2
Soil	2	16	219.3 ± 19.0	8	156.2 ± 15.1	8	208.6 ± 43.1
Soil	3	16	205.8 ± 19.8	8	147.0 ± 16.2	8	222.6 ± 38.6
Water (DGT)	1	16	24.3 ± 3.7	8	79.4 ± 23.3	8	85.2 ± 32.0
Water (DGT)	3	16	162.1 ± 22.8	8	137.5 ± 36.7	–	–
Rice – roots	1	16	71.5 ± 9.4	8	46.0 ± 6.0	8	67.9 ± 21.5
Rice – stalk	1	16	43.0 ± 6.3	8	36.7 ± 3.1	8	18.2 ± 3.9
Rice – leaves	1	16	44.9 ± 9.3	8	37.0 ± 3.9	8	21.3 ± 3.4
Rice – roots	2	16	47.7 ± 5.1	8	41.4 ± 6.3	8	78.2 ± 14.7
Rice – stalk	2	16	10.0 ± 2.2	8	9.4 ± 1.5	8	9.2 ± 1.6
Rice – leaves	2	16	15.4 ± 1.3	8	11.5 ± 1.0	8	18.9 ± 2.0
Rice – grain	2	16	11.9 ± 2.4	1	10.8 ± 0.0	8	12.1 ± 2.6
Rice – roots	3	16	110.1 ± 8.7	8	70.4 ± 7.5	8	105.2 ± 20.6
Rice – stalk	3	16	2.7 ± 0.5	8	3.2 ± 0.5	8	1.5 ± 0.3
Rice – leaves	3	16	14.8 ± 0.9	8	10.8 ± 1.6	8	14.0 ± 1.0
Rice – grain	3	16	3.8 ± 0.4	8	2.9 ± 0.2	8	3.9 ± 0.7

mass. 0.05–0.10 g of soil sample were analyzed for THg concentration as described above.

Mercury speciation analysis was performed in soil samples corresponding to the EW fields on the June/July and August sampling periods (N = 16 soil cores) to determine the concentration of MeHg in the samples. MeHg concentration was determined by species specific isotope dilution and gas chromatography coupled to inductively coupled plasma mass spectrometry (SSID-GC-ICP-MS) after a microwave assisted digestion in HNO_3 6 M (Monperrus et al., 2008; Feng et al., 2016).

2.2.3. Rice plants

For each sampling point at each sampling period, 5–10 rice plants were collected and stored in ziplocked bags at −20 °C until analysis. Plants were allowed to thaw before analysis and were rinsed with distilled water to remove surface contamination. After cleaning, we used stainless-steel scissors to separate each plant in 4 parts: roots, stalk, leaves and grains. For each sampling point and sampling event, each of the plant parts for the 5–10 plants collected was pooled together. They were oven-dried to constant mass at 50 °C and then ground to a homogeneous powder with an impactor mill (Freezer Mill 6850, SpexCertiPrepH Inc., Metuchen, NJ, USA) operating at liquid nitrogen temperature. Approximately 0.50 g of powdered plant samples were analyzed for THg concentration as described above.

A total of N = 96 rice plant samples (5–10 plants per sample) were collected, separated in parts, and analyzed for THg, corresponding to each sampling point for the first, second and third sampling events. Since rice plants in the first sampling event (June/July) were in the vegetative phase and only for the second (August) and third (September) sampling events, plants presented the panicle, THg concentration in rice grains was only analyzed on the last two sampling events (Table 1).

Rice plant samples from the first and third sampling periods from the EW fields (N = 16 plant samples) were analyzed for Hg speciation following the same method as described above (SSID-GC-ICP-MS) after a microwave assisted digestion in HNO_3 6 M (Monperrus et al., 2008; Feng et al., 2016). Nonetheless, we could not perform speciation analyses on n = 3 and n = 1 stalk plant tissue, from the first and third sampling periods, respectively, due to low sample quantity.

2.2.4. Fauna samples

Fauna samples were collected by placing funnel traps for 24 h in each of the sampling points on each sampling period or with aerial nets. Different taxa of invertebrates and small vertebrates were collected (Table 3) and individuals were stored in separate ziplocked bags at −20 °C until analysis.

Upon analysis, samples were rinsed with distilled water to remove external contamination. Then, for each of the individuals we recorded the following biometrical measurements: body weight (mg) and total body length (mm). For crayfish (*Procambarus clarkii*) samples, individuals were dissected and hepatopancreas and abdominal muscle tissue were collected for mercury analyses. For each of the dragonfly species collected (*Sympetrum fonscolombii* and *Trithemis annulata*), 2–3 individuals were pooled in each sample to attain sufficient mass for mercury analyses (Table 3). Finally, prior to total mercury determination animal samples were freeze-dried until constant mass and ground to a homogeneous powder using a mortar and pestle or an impactor mill (Freezer Mill 6850, SpexCertiPrepH Inc., Metuchen, NJ, USA). Total mercury analysis was performed as described above.

For carbon and nitrogen stable isotopic analyses, the powdered samples were lipid extracted using 2:1 chloroform:methanol solution following the Folch method (Folch et al., 1957). From crayfish, only abdominal muscle samples were analyzed for carbon and nitrogen stable isotopes. Due to low sample quantities and to avoid sample losses, dragonfly samples were not lipid extracted. Then, 0.30–0.35 mg of powdered samples were placed in tin cups and analyzed via a Thermo-Finnigan Flash 1112 elemental analyzer (CE Elantech, Lakewood, NJ, USA) coupled to a Delta-C isotope ratio mass spectrometer via a CONFLO III interface (Thermo Finnigan MAT, Bremen, Germany). Stable isotope signatures of C and N are expressed using the standard delta notation in ‰ relative to the Vienna Pee Dee Belemnite ($\delta^{13}C$) and atmospheric N_2 ($\delta^{15}N$).

A total of N = 204 fauna samples corresponding to 10 different species were collected among all the rice fields sampled (Table 3). Since low samples sizes were obtained, for the statistical analyses, samples from the three sampling periods were analyzed together for each agricultural type.

Finally, mercury speciation analyses were performed on the subset of samples corresponding to the three sampling periods of EW fields (N = 22 fauna samples). Mercury species were determined by means of same method described above (SSID-GC-ICP-MS) (Monperrus et al., 2008; Feng et al., 2016) after a microwave assisted digestion in HNO_3 6 M, in the case of insect samples, or after a tetramethylammonium hydroxide (TMAH) microwave assisted digestion, in the case of small vertebrates and crayfish samples (Table 3).

2.2.5. Quality control

To assess the accuracy of measurements we analyzed several matrix

matched (certified) reference materials every 10–15 samples for THg determination and MeHg and iHg analyses as detailed in Table S1 and Table S2: DORM-4 (fish protein), DOLT-5 (dogfish liver), NIES-13 (human hair), IAEA456 (marine sediment), BCR 414 (zooplankton), ERM CE-464 (tuna fish), GBW 08508 (rice flour), BCR 281 (rye grass). The recoveries ranged from 93 to 101 %, therefore, no corrections were applied to the THg values of the samples prior to statistical analyses. MeHg concentrations detection limits for speciation analysis (ng/g dw) were of 0.1 for sediments, 0.4 for insects, 0.5 for fish, frogs and crayfish, 2.6 for feathers, and 0.1 for plant tissues. For MeHg and iHg measurements all the samples were analyzed in triplicate.

For stable isotope analyses of N and C in fauna samples, international standards (IAEA CH_6 (42 % of C), IAEA CH_7 (87 % of C), and USGS 24 (100 % of C) for ^{13}C and IAEA N_1 and IAEA N_2 (with 21 % of N) and IAEA NO_3 (13.8 % of N) for ^{15}N) were applied every 12 samples to calibrate the system. Replicate assays of standards indicated analytical measurement errors of ±0.1 ‰ and ±0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.3. Data treatment and statistical analyses

Data were visually inspected for the presence of outliers and normality. Total mercury concentrations and MeHg concentrations were log-transformed to approximate the values to a normal distribution. All analyses were performed using R v.4.0.3 (R Core Team, 2020). Statistically significant threshold was set at $\alpha = 0.05$. Model fits were assessed by visual inspection of residual analyses.

2.3.1. THg concentrations in water, soil and rice plants

For water and soil compartments, linear mixed effects models were fit using the *lme4* package (Bates et al., 2015) to evaluate the effect of agricultural management (CW, EW, ED) and sampling period (June/-July, August, September for the soil model, and June/July, September for the water model) as fixed effects on the log THg concentrations determined. The interaction between agricultural management and sampling period was included as fixed effect. Rice field ID was included as a random effect.

For the rice plant samples, linear mixed effects models were fit to evaluate the effect of agricultural management (CW, EW, ED), sampling period (June/July, August, September), plant part (roots, stalk, leaves and grain) on the log THg concentrations. Furthermore, the interaction between plant part and sampling period was included as fixed effect. Rice field ID was included as a random effect.

Pearson correlation tests were performed to evaluate the relationship between THg concentrations in soil and each of the plant parts, including all the periods sampled considering all the agricultural types together.

2.3.2. THg concentrations and stable isotopes of C and N in fauna samples

Due to low sample sizes of collected individuals from each of the rice field types (CW, EW and ED) in each sampling period (Table 3), we pooled the data from the three sampling events for each agricultural management type.

For each of the species collected, differences in $\delta^{13}C$, $\delta^{15}N$ and THg concentration among agricultural types was tested using ANOVA with agricultural type as factor, if species were present on the three rice field types. Post-hoc significant differences were further evaluated by using Tukey's honest significant test. If only present in two rice field types, t-tests were used to evaluate differences in $\delta^{13}C$, $\delta^{15}N$ and THg among agricultural management types. For these tests, we used only observations with $n > 1$ (Table 3).

Using the $\delta^{15}N$ values we estimated the mean ± SD trophic level (TL) for each species sampled according to the following equation (Post, 2002):

$$TL_{consumer} = \left(\frac{\delta^{15}N_{consumer} - \delta^{15}N_{base}}{\Delta^{15}N}\right) + TL_{base}$$

where, $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ value for the consumer, $\delta^{15}N_{base}$ and TL_{base} represent the $\delta^{15}N$ value and the trophic level of the organism considered to set the baseline, respectively, and $\Delta^{15}N$ is the trophic discrimination factor. As baseline organism we selected the coleopteran *Hydrophilus pistaceus*, which feeds on aquatic plants. Thus, $TL_{base} = 2.0$, as it is a primary consumer (Post, 2002), and $\delta^{15}N_{base}$ is the mean value of $\delta^{15}N$ for *H. pistaceus* in each of the agricultural management types (Table 3). $\Delta^{15}N$ was set at 3.4, for aquatic food webs (Vander Zanden and Rasmussen, 2001).

Log THg concentrations and trophic levels estimated from $\delta^{15}N$ were used to estimate the trophic magnification factor (TMF) following Borgå et al. (2012). Briefly, log THg was regressed against TL obtaining the parameters for the intercept (a) and slope (b) in the equation Log [THg] $= a + b$TL. Then, the TMF indicating the rate of increase in concentration of THg with trophic position was calculated as $TMF = 10^b$, where b is derived from the previous linear regression. TMF >1 indicates magnification of the pollutant along the food web considered (Borgå et al., 2012; Conder et al., 2012).

2.3.3. MeHg proportions in soil, plants and fauna

Mercury speciation analyses for plant and fauna samples in EW fields on the first and third sampling events, and for soil samples in EW fields on the first and second sampling events were used to calculate the

Table 2
Sample size (N) and summary statistics (mean ± SD) for each of the parameters ([THg], [MeHg] and MeHg/THg) in soil, rice plant parts and fauna samples from ecological wet rice fields. For soil and rice plant samples, data are shown for each sample period. For fauna samples data were pooled among the three sample periods (see text for details). For fauna samples, N is the number of individuals analyzed, except for *S. fonscolombii*, where N is the number of pools (2–3 individuals) analyzed.

Sample type	Species	Sample Period	N	THg (ng/g dw)	MeHg (ng/g dw)	MeHg/THg (%)
Soil		1	8	157.9 ± 49.8	1.3 ± 0.02	0.8 ± 0.3
Soil		2	8	156.2 ± 42.8	1.0 ± 0.01	0.7 ± 0.3
Rice – roots	*O. sativa*	1	8	46.0 ± 16.9	6.4 ± 5.3	16.7 ± 9.8
Rice – stalk	*O. sativa*	1	5	32.1 ± 7.4	14.9 ± 0.2	46.4 ± 15.4
Rice – leaves	*O. sativa*	1	8	37.0 ± 11.1	10.5 ± 0.4	28.3 ± 7.5
Rice – roots	*O. sativa*	3	8	70.4 ± 21.3	3.8 ± 0.3	5.7 ± 4.0
Rice – stalk	*O. sativa*	3	7	3.6 ± 1.1	0.8 ± 0.5	24.6 ± 14.0
Rice – leaves	*O. sativa*	3	8	10.8 ± 4.5	0.7 ± 0.01	8.4 ± 6.5
Rice – grain	*O. sativa*	3	8	2.9 ± 0.6	1.9 ± 0.05	65.6 ± 17.3
Fauna	*C. carassius*		1	244.0 ± 0.0	181.9 ± 0.0	74.5 ± 0.0
Fauna	*H. pistaceus*		2	144.9 ± 2.7	60.6 ± 1.4	41.7 ± 9.5
Fauna	*P. clarkii*		9	368.2 ± 117.4	357.7 ± 7.7	97.5 ± 9.9
Fauna	*R. perezi*		5	220.0 ± 75.1	122.7 ± 2.0	57.6 ± 14.9
Fauna	*S. fonscolombii*		2	325.2 ± 62.0	254.2 ± 4.3	78.4 ± 12.5

Table 3
Sample size (N) and summary statistics (mean ± SD) for each of the parameters ($\delta^{15}N$, $\delta^{13}C$, [THg]) in each of the species collected during the 2017 rice cultivation season. Data was pooled together for the three sampling events and for each of the agricultural management types. Sample sizes refer to individuals collected and analyzed except for *S. fonscolombii* and *T. annulata*, that refer to pools of 2–3 individuals (see text for details). Statistically significant differences for each species and each parameter among field types ($p < 0.05$) in means from samples with $N > 1$ were evaluated.

Species	Conventional Wet (CW)					Ecological Wet (EW)					Ecological Dry (ED)				
	N	THg (ng/g dw)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	TL	N	THg (ng/g dw)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	TL	N	THg (ng/g dw)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	TL
C. carassius	1	286.8 ± 0.0	−25.7 ± 0.0	13.7 ± 0.0	3.5 ± 0.0	2	287.4 ± 61.3	−26.7 ± 0.0	12.5 ± 0.0	3.4 ± 0.0	0	–	–	–	–
C. lateralimarginalis	0	–	–	–	–	2	220.8 ± 29.8	−28.2 ± 2.1	11.7 ± 0.6	3.1 ± 0.2	0	–	–	–	–
E. sticticus	1	447.4 ± 0.0	–	–	–	0	–	–	–	–	0	–	–	–	–
H. pistaceus	4	175.4 ± 101.7	−29.9 ± 1.7	8.5 ± 0.7	2.0 ± 0.2	3	173.4 ± 49.4	−31.0 ± 2.7	7.9 ± 0.7	2.0 ± 0.2	1	145.6 ± 0.0	−29.2 ± 0.0	5.9 ± 0.0	2.0 ± 0.0
M. angillicaudatus	6	149.0 ± 17.5	−26.3 ± 0.8	11.6 ± 0.7	2.9 ± 0.2	0	–	–	–	–	0	–	–	–	–
P. clarkii	104	256.9 ± 129.6[a]	−28.5 ± 2.8	10.4 ± 1.5	2.6 ± 0.5	20	333.6 ± 85.4[b]	−27.7 ± 1.4	11.2 ± 1.8	3.0 ± 0.5	0	–	–	–	–
P. parva	0	–	–	–	–	0	–	–	–	–	1	398.8 ± 0.0	−23.5 ± 0.0	13.3 ± 0.0	4.2 ± 0.0
R. perezi	13	346.1 ± 68.2	−23.2 ± 1.5	11.1 ± 0.7	2.8 ± 0.2	12	302.3 ± 90.7	−23.5 ± 1.8	10.7 ± 2.4	2.8 ± 0.7	5	395.7 ± 92.3	−23.5 ± 1.3	9.5 ± 2.3	3.1 ± 0.7
S. fonscolombii	5	323.3 ± 52.3	−27.5 ± 1.7	9.6 ± 2.1	2.3 ± 0.6	3	327.1 ± 52.0	−27.3 ± 0.8	9.3 ± 1.7	2.4 ± 0.5	1	433.5 ± 0.0	−26.0 ± 0.0	6.3 ± 0.0	2.1 ± 0.0
T. annulata	0	–	–	–	–	2	350.7 ± 75.3	−27.3 ± 1.3	10.8 ± 1.1	2.8 ± 0.3	2	372.2 ± 33.6	−22.9 ± 3.5	10.2 ± 1.8	3.3 ± 0.5

[a, b] Statistically significant differences ($p < 0.05$).

proportion of MeHg in samples relative to THg. A descriptive summary of the results is shown in Table 2. Differences in MeHg concentration among the first and second sampling periods for soil samples were evaluated with t-tests.

For rice plant samples, differences in MeHg to THg proportion were evaluated in the third period for all the plant parts (roots, stalk, leaves, grain) using ANOVA with plant part as factor, since grain samples were only available for the third period. Additionally, a linear model was fitted to evaluate the effect of the plant part, sampling event and their interaction on MeHg using only data for roots, leaves and stalks.

MeHg concentrations in fauna from EW rice fields were used to estimate the trophic magnification factor (TMF) for this food web, as described before.

3. Results

3.1. Physicochemical parameters of water

Results of the physicochemical measurements are reported in Table S3. Mean pH values ranged from 7.27 to 7.55 for CW fields, from 4.99 to 7.61 for EW fields, and from 6.60 to 6.86 for ED fields. For both CW and ED fields pH values remained constant throughout the rice

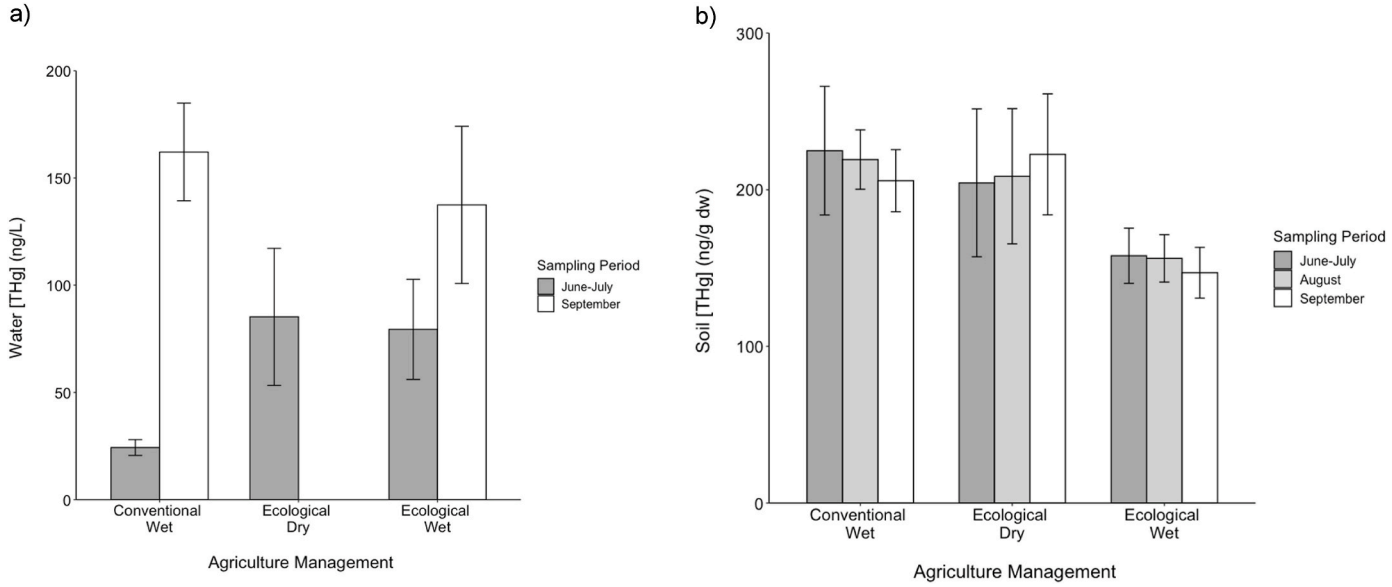

Fig. 1. (a) Water THg concentrations (ng/L) obtained by DGT technique on three sampling periods (June/July, August, September), and (b) soil THg concentrations (ng/g dw) from cores obtained on three sampling periods (June/July, August, September) during the 2017 rice cultivation season from rice fields farmed using three different agricultural management types (Conventional Wet, Ecological Dry, Ecological Wet). Error bars depict the SE of the mean. Significant differences in water THg levels were detected among CW-ED and CW-EW for the first sampling period (June/July) and between the first and third sampling periods in both CW and EW rice fields. Soil THg levels did not differ significantly among agricultural management types or sampling periods.

cultivation season, whereas in EW fields, pH decreased over the rice cultivation season. Temperature values ranged between 24 and 31 °C in all field types, without any clear trends throughout the rice cultivation season for any of the field types considered. In addition, dissolved oxygen in water, salinity, and conductivity presented low variation within the study period (Table S3).

3.2. THg concentrations in water, soil and rice plants

For THg concentrations in water by DGT, those ranged from 24 to 162 ng/L (Table 1). A significant effect of agricultural types considered for the first sampling event was found ($F_{2,29} = 11.01$, $p = 0.0003$). Post-hoc pairwise comparisons showed significant differences between CW and EW, and CW and ED, but no differences between EW and ED (CW-EW: $p = 0.027$, CW-ED: $p = 0.028$, EW-ED: $p = 1.00$; Fig. 1a). Regarding the third sampling period no differences in water Hg levels between CW and EW fields were detected ($F_{1,21} = 0.87$, $p = 0.36$). Significant differences were observed in mercury concentration in water between the first and the third sampling period in CW ($F_{1,29} = 74.29$, $p < 0.0001$) from 24.3 ± 3.7 to 162.1 ± 22.8 ng/L. However, although an increase in THg levels was observed in EW fields from the first to the third sampling periods (Table 1), no significant differences were detected ($F_{1,13} = 2.23$, $p = 0.16$; Fig. 1a).

The linear mixed model for the soil samples did not show any significant differences in THg levels among agricultural types ($F_{2,5} = 0.16$, $p = 0.85$) or sampling periods ($F_{2,81} = 1.09$, $p = 0.34$). The interaction term was not significant ($F_{4,81} = 0.52$, $p = 0.72$; Fig. 1b).

Regarding the rice plant samples, we found significant differences in THg levels among plant parts ($F_{3,311} = 166.20$, $p < 0.0001$). Pairwise comparisons showed significant differences among all the plant parts pairs (in all cases, $p < 0.0001$), except between stalk and grain ($p = 1.00$), with THg concentrations in roots > leaves > stalk ≈ grain (Fig. 2). The model including only the data from roots, leaves and stalk samples for the three sampling periods showed a significant interaction between the sampling period and the plant parts ($F_{4,240} = 56.84$, $p < 0.0001$). Models for each of the plant parts, showed a significant increase in THg concentration in roots over time ($F_{2,78} = 16.41$, $p < 0.0001$), while THg levels decreased in stalks and leaves (stalks: $F_{2,78} = 108.37$, $p < 0.0001$; leaves: $F_{2,74} = 42.80$, $p < 0.0001$). No significant differences in THg levels for each plant part were found among agricultural types (in all cases, $p > 0.10$). Regarding grain samples a significant decrease in THg concentration was observed between the second and third sampling events ($F_{1,45} = 70.95$, $p < 0.0001$), and no significant differences in rice grain THg levels were detected among agricultural management types ($F_{2,5} = 0.010$, $p = 0.99$).

Pearson correlation tests between soil and plant parts THg concentrations showed significant positive correlations between soil and roots, and soil and rice THg levels (soil-roots: $r = 0.47$, $p < 0.0001$; soil-grain: $r = 0.37$, $p < 0.0001$), but not between soil and stalk or leaves THg concentrations (soil-stalk: $r = 0.099$, $p = 0.35$; soil-leaves: $r = 0.16$, $p = 0.13$; Fig. S2).

3.3. THg concentrations and stable isotopes of C and N in fauna samples

A summary of the species collected, THg levels, $\delta^{15}N$ and $\delta^{13}C$ values, and estimated TL, for each agricultural management type is shown in Table 3. THg values ranged from 149.0 ± 17.5 to 447.4 ± 0.0 ng/g dw in fauna samples from CW fields, from 173.4 ± 49.4 to 350.7 ± 75.3 ng/g dw in fauna from EW fields and from 145.6 ± 0.0 to 433.5 ± 0.0 ng/g dw in ED fields (Table 3). Likewise, differences in ranges of $\delta^{15}N$ and $\delta^{13}C$ values were observed in for the different fauna collected at each of the field types considered. Significant differences in THg, $\delta^{13}C$ and $\delta^{15}N$ are indicated in Table 3.

For the three types of agricultural management practices (CW, EW, ED) trophic magnification factors considering the species collected in each field type was TMF > 1 (CW: 1.25; ED: 1.53; EW: 1.45), indicating an effective biomagnification of Hg along food webs in each agricultural management type (Fig. 3).

3.4. MeHg proportions in soil, plants and fauna from EW fields

MeHg to THg proportions in soil samples ranged from 0.4 to 1.3 % (Table 2). T-test showed no significant differences between first and second sampling period in MeHg to THg propotions in soils ($t = 1.40$, $p = 0.18$).

In rice plant samples, MeHg to THg proportions ranged from 5 to 64

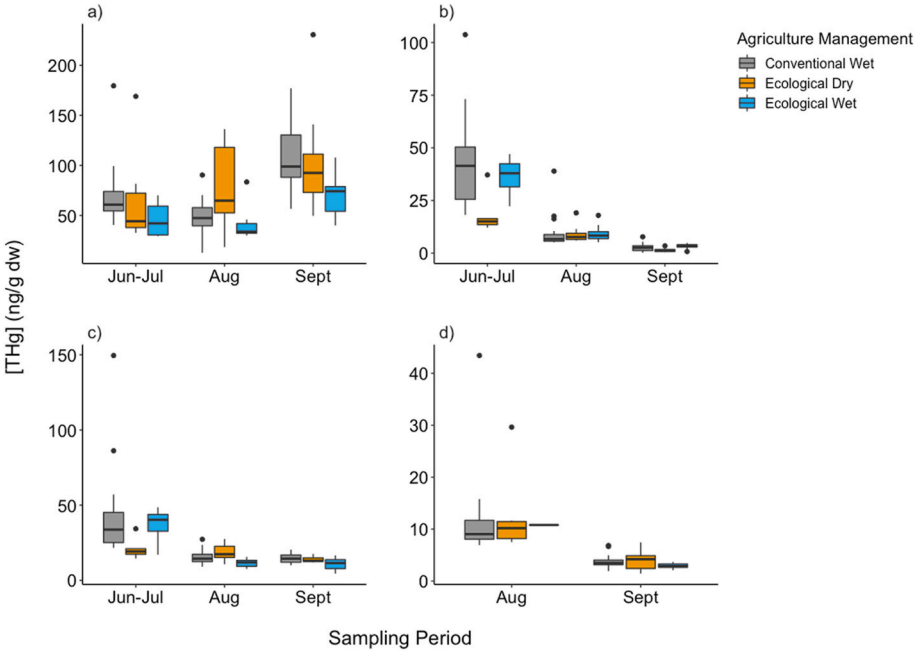

Fig. 2. THg concentrations (ng/g dw) in rice plant parts: (a) roots, (b) stalk, (c) leaves, (d) grain, in three sampling periods over the rice cultivation season from 2017 in the Ebro Delta. Samples from fields farmed using different agricultural management practices (Conventional Wet, Ecological Dry, Ecological Wet) were analyzed.

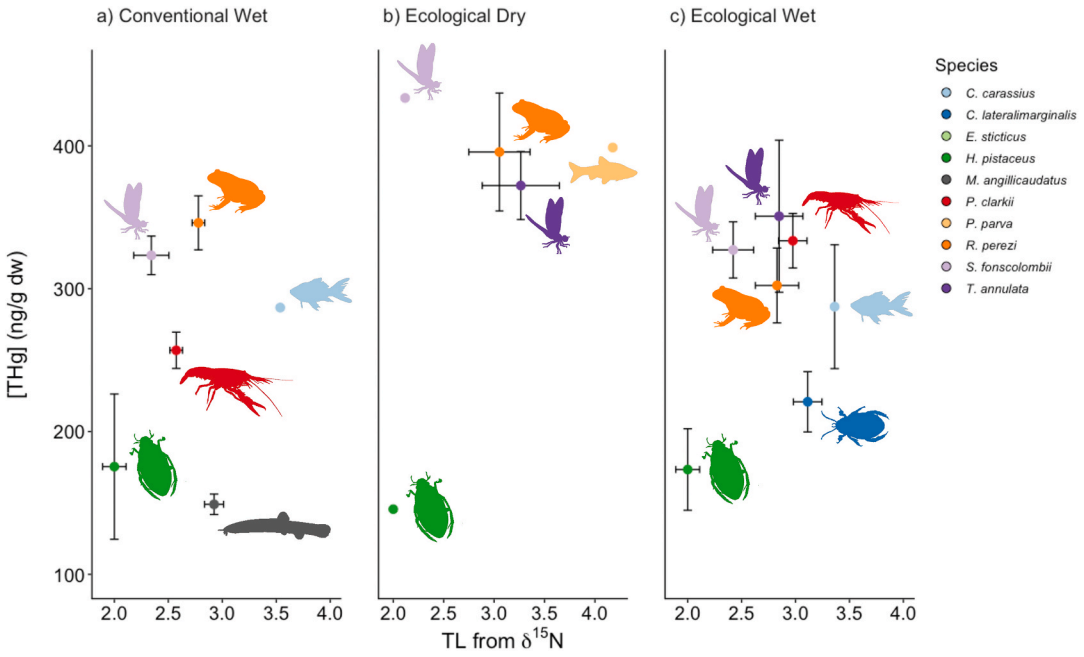

Fig. 3. THg concentrations vs. trophic level (TL) in fauna samples for the three types of agricultural management fields: (a) Conventional Wet, (b) Ecological Dry and (c) Ecological Wet. Trophic magnification factors (TMF) for THg were > 1 in all three rice field types indicating an effective biomagnification of mercury along the foodweb.

% for the first sampling period, and from 2 to 87 % in the third sampling period, depending on the plant part (Table 2). We detected significant differences in MeHg proportions among plant parts on the third period of sampling ($F_{3,27} = 44.82$, $p < 0.0001$). Post-hoc comparisons between plant parts showed differences among MeHg proportions between grain and the other parts of the plant, and between stalk and roots (roots-grains: $p < 0.0001$; roots-leaves: $p = 0.97$; roots-stalk: $p = 0.021$; leaves-grains: $p < 0.0001$; leaves-stalk: $p = 0.06$; stalk-grains: $p < 0.0001$), with grain accumulating higher MeHg proportions compared to the other parts (Table 2). The linear model including only data for roots, leaves and stalks for the first and third period, detected significant differences in MeHg to THg proportions among plant parts ($F_{2,\ 38} = 18.71$, $p < 0.0001$) and between sampling events ($F_{1,\ 38} = 34.00$, $p < 0.0001$). The interaction between plant part × sampling period was not significant ($F_{2,38} = 1.31$, $p = 0.28$).

For the rice-fields fauna samples, the proportion of MeHg to THg ranged from 35 to 112 %, depending on the species (Table 2). MeHg concentrations differed significantly among species ($F_{4,17} = 17.94$, $p < 0.0001$) but, although positive, the correlation between MeHg concentration and TL was not significant ($r = 0.30$, $p = 0.16$), possibly due to a low statistical power. The TMF for MeHg was of 1.94.

4. Discussion

The presence of a chlor-alkali industry along the Ebro river has been considered as an important source of Hg in water, soil and biota (Navarro et al., 2009; Carrasco et al., 2011a, 2011b; Cotín et al., 2011, 2012; Sánchez-Fortún et al., 2020). Rice fields environments have been extensively studied as mercury hotspots (Zhao et al., 2020; Tang et al., 2020), since environmental conditions are favorable for microbial growth and Hg^{2+} methylation (Rothenberg et al., 2014; Marvin-Di-Pasquale et al., 2014), and thus, transference to rice plants (Meng et al., 2011, 2014; Rothenberg and Feng, 2012; Wang et al., 2018) and biota inhabiting the rice fields (Ackerman and Eagles-Smith, 2010; Ackerman et al., 2010; Sánchez-Fortún et al., 2021; Du et al., 2021). Particularly, the distribution of Hg in rice plants has been of interest since its ingestion might pose one of the main entrance pathways of mercury for humans, especially in areas where rice is a staple-food (Rothenberg et al., 2014; Liu et al., 2019).

Moreover, agricultural management of the fields might vary the physicochemical properties of the rice field environment and thus affect mercury bioavailability and transformation (Ullrich et al., 2001; Tang et al., 2018, 2020), and ultimately its accumulation in plants and fauna (Ackerman et al., 2010; Tanner et al., 2018; Sánchez-Fortún et al., 2021). Among factors influencing Hg bioavailability in rice fields environment, hydroperiod has been studied extensively (e.g., Marvin-Di-Pasquale et al., 2014; Rothenberg et al., 2014, 2016; Tanner et al., 2018). In the present study we have evaluated how different agricultural management techniques regarding the usage of pesticides (conventional vs. ecological) and the hydroperiod (wet vs. mild AWD) influence the Hg availability for different compartments in the rice fields environments, namely soil, water, rice plants and fauna, and how it varies along the rice growing season.

4.1. Physicochemical parameters of water

Physicochemical characteristics of the environment have been regarded as one of the main drivers of mercury transformation throughout its biogeochemical cycle, including methylation and bioaccumulation processes (Ullrich et al., 2001; Celo et al., 2006; Lavoie et al., 2013; Lescord et al., 2019). In rice fields, parameters such as pH, oxygen dissolved in water, temperature, salinity and conductivity are known to influence mercury methylation (Ullrich et al., 2001; Rothenberg and Feng, 2012). In our study, we have measured these parameters in each of the sampling periods (Table S3).

In the fields under study mean pH values ranged from 7.27 to 7.55 for CW fields, 4.99–7.61 for EW fields, and 6.60–6.86 for ED fields. Likewise, other studies in rice paddies have detected similar pH values in paddies' water (Rothenberg and Feng, 2012; Zhao et al., 2016a; Sánchez-Fortún et al., 2021). pH values for CW and ED remained constant throughout the rice cultivation season, whereas in EW fields, pH decreased along the rice growing season. Nonetheless, with the available data the measurements of pH do not present a relationship with THg concentrations in CW, EW or ED fields, or with MeHg in EW fields for the three sampling periods considered. Thus, in our case, pH might not be mainly influencing mercury dynamics in the rice field compartments

studied, and in fact, although in some cases pH conditions might affect Hg methylation (Ullrich et al., 2001), the generalization of these conditions for MeHg production is unclear (Tang et al., 2020) with different environments showing Hg methylation in a relatively wide range of pH conditions (e.g., Braaten et al., 2014). Regarding temperature values, measurements ranged between 24 and 31 °C, and although temperature might have influence on Hg methylation and its accumulation in organisms (e.g., Ullrich et al., 2001), we do not have enough data to evaluate this. Nonetheless, other studies in rice fields (e.g., Rothenberg and Feng, 2012) suggested that temperature might not be a main driver of Hg methylation in these environments. Likewise, dissolved oxygen in water, salinity, and conductivity present low variation within the study period, which might indicate a little influence on mercury dynamics in our study.

However, other parameters that have not been measured in the present study such as dissolved organic matter (DOC) abundance and composition, or the presence of electron acceptors as sulfate or iron, which do have an influence in availability of Hg to bacterial methylators and biomethylation processes (Hall et al., 2008; Bravo and Cosio, 2020; Li et al., 2022), might be playing a role on the results observed. Thus, we do have limited data available on physicochemical parameters, and in this regard, more experimental and *in-situ* studies need to be performed to further evaluate the consequences of agricultural management practices on environmental physical and chemical properties, and their relationship with Hg cycling in complex environments such as rice paddies (Tang et al., 2020).

4.2. THg concentrations in water, soil and rice plants

Compared to the other environmental compartments studied, low Hg concentrations in rice fields' water were detected in all the three agricultural types studied (range: 24–162 ng/L), which might be introduced in the rice paddies through irrigation. Indeed, rice fields in the area are irrigated using the water from the Ebro river, which transports significant amounts of Hg due to the legacy mercury pollution from industries along the river (Terrado et al., 2006; Carrasco et al., 2008; Palanques et al., 2020).

Differences among rice field types in THg concentration in water from the first sampling event were detected between conventional and ecological fields, despite the hydroperiod conditions applied to the ecological fields, with higher Hg concentrations in water from EW or ED than CW fields. Nonetheless, these differences might arise from the distance among the rice fields studied (Fig. S1), and thus, irrigation water sources, rather than a particular influence of the usage of pesticides and fertilizers.

The Hg concentration in rice paddies water increased for both CW and EW rice fields from the first sampling period to the third. Thus, it might indicate the continuous load of Hg into the rice fields coming from the water sourced to irrigate the fields (Terrado et al., 2006; Palanques et al., 2020). Unfortunately, in ED we could not evaluate the difference in Hg concentration in water between the first and third sampling periods, although we would expect a similar trend in THg concentration over time.

Soil samples Hg concentration did not differ statistically among agricultural types, although EW fields presented lower Hg levels in soil compared to both ED and CW. These differences, even if not statistically significant, could be an effect of particularities within EW fields compared to ED or CW fields that have not been considered in the present study (e.g., irrigation water source, methylation rate within the fields and transfer of MeHg to other environmental compartments; Zhou et al., 2015; Tang et al., 2020). Moreover, we did not found differences on Hg concentrations in soils among the three sampling periods (Fig. 1b; Table 2). Other studies have found similar results (e.g., Rothenberg and Feng, 2012; Zhao et al., 2016b), with THg levels in soil maintained throughout the rice cultivation season. Nonetheless, although THg concentration is maintained throughout the rice growing season, the fraction of Hg bound to organic-matter in paddy soils might change with rice plant growth, making it readily available for methylation and uptake by rice plants (Zhou et al., 2015). Based on our results, the application of fertilizers and pesticides does not seem to influence THg concentrations in soil samples (Table 1), although other studies point out an influence of the application of fertilizers to Hg concentrations in agricultural soils (Tang et al., 2018, 2019). Furthermore, both continuous flooding and AWD techniques do not seem to have a great impact on soil THg concentrations (Rothenberg et al., 2016). Thus, although mercury apportionment in the rice fields is continuous through irrigation water (Dai et al., 2013), soil mercury concentrations might depend on different factors related to the geochemistry of the soil (Ullrich et al., 2001; Rothenberg and Feng, 2012), and their combination do maintain soil THg levels throughout the rice cultivation season.

Regarding THg concentrations in rice plants (Fig. 2), our results show differences among different plant parts, which reflects the differential capacity of plant tissues to accumulate this metal (Meng et al., 2010; Zhang et al., 2010; Tang et al., 2020; Liu et al., 2021), with roots presenting higher THg concentrations than the aerial parts of the plant (roots > leaves > stalk ≈ grain). Nonetheless, THg concentrations in plant parts showed an increase for roots and a decreasing trend in stalk, roots and grains over the rice cultivation season. Decrease in THg concentrations in grain, stalk and leaves is probably due to his dilution because of the increase in biomass with growth (Shu et al., 2016). However, the increase in THg concentration observed in roots might be related to the capacity of roots to sequestrate inorganic Hg using phytochelatins in the cytosol of root cells and transporting it into the vacuoles (Krupp et al., 2009; Tang et al., 2020); thus, the increase in THg in roots is mainly due to an increase in inorganic Hg forms rather than MeHg.

Furthermore, our results are in accordance with other similar studies (Meng et al., 2010, 2011, 2014; Zhang et al., 2010; Hang et al., 2018) in which higher concentrations in roots and grain were related to Hg levels in soil. In this study, THg levels in soil samples were correlated with those of roots and grain, indicating that soil Hg might be the main Hg source for these two rice tissues (Meng et al., 2010). However, soil THg concentrations did not correlate with THg in leaves or stalk. In this case, field studies in Hg-polluted sites (Meng et al., 2010, 2011, 2012) have also confirmed that aerial parts (leaves and stalks) from rice plants, can accumulate Hg from the air (Marrugo-Negrete et al., 2016). Furthermore, experimental studies using Hg stable isotopes have found that leaves tend to accumulate mercury from the air through the stomata (Tang et al., 2021). Thus, the uncoupling between THg concentrations in leaves and stalk, and soil samples found in our study might be due to the incorporation of Hg from the air on rice plant tissues.

We did not find any significant differences on THg accumulation by each plant part or their trend on THg concentrations over the rice plant growth period among the different agricultural practices studied, implying that neither hydroperiod nor the application of fertilizers and pesticides had an influence on THg levels in the different plant parts. Regarding hydroperiod, Tanner et al. (2018) have reported similar results, with AWD growth rice plants presenting THg concentrations similar to those growth in continuously flooded conditions, but when considering MeHg concentrations they were lower in plants from AWD treatments. In addition, Rothenberg et al. (2016) reported both decreases in THg and MeHg accumulation by rice plants under AWD conditions compared to continuously flooded conditions, although declined concentrations were dependent on the severity of AWD treatment employed. Also, application of fertilizers and pesticides does not seem to influence THg accumulation in rice plants, although we have a limited sample size and exactly applications were unknown. Moreover, a recent review by Tang et al. (2019) points out that some fertilizers might affect MeHg production in rice paddies soils and thus the overall levels of MeHg in plants. Unfortunately, we did not measure MeHg concentrations for all the agricultural management practices, and thus, we could not evaluate whether in our study MeHg concentrations in rice

plants was affected by the alternating wetting and drying management in comparison to continuously flooded conditions or by ecological vs. conventional treatments.

4.3. MeHg concentrations in soil and rice plants in EW fields

Regarding MeHg concentrations in soil from ecological wet fields, they did not differ between the first and second sampling periods, which contrast with other studies that found an increase in MeHg concentration in soil samples over rice growth (Zhou et al., 2015). However, our data does not range the entire rice plants growth, and Zhou et al. (2015) observed the major increase in MeHg in paddy soil samples during the heading stage of the rice plants, which were not attained during the second sampling we performed.

When observing how MeHg proportions were distributed within the plant from ecological wet fields, the grain presented a higher MeHg to THg proportion (66 %) than the other plant parts, followed by stalk (25 %) and leaves (8 %) and finally, roots (6 %). Conversely, roots did accumulate mainly inorganic mercury (Table 2). It has been suggested that root uptake of heavy metal by plant from the soil could be controlled by phytochelatins. These peptides composed of cysteines are chelating agents that would serve to sequester inorganic Hg and keep it stored in vacuoles (Meng et al., 2010, 2014; Zhang et al., 2010; Rothenberg et al., 2014; Turull et al., 2017b). Thus, roots can act as a barrier in the translocation of Hg^{2+} to aerial parts but not for MeHg (Meng et al., 2010, 2011; Zhang et al., 2010), that could explain why MeHg is mainly found in grain in several studies (Meng et al., 2010, 2011, 2014; Zhang et al., 2010; Rothenberg et al., 2011), including the present study. Comparing the first and third sampling period, in roots, leaves and stalk, we observe a decrease in MeHg concentrations significant only for leaves and stalk but not for roots. This trend might be due to a biodilution of the metal with an increase in biomass over plant growth (Shu et al., 2016) and *in vivo* demethylation of MeHg in the aerial parts of the plants (Xu et al., 2016; Strickman and Mitchell, 2017; Liu et al., 2021). In the case of roots, a selective transport of MeHg to the aerial parts (Meng et al., 2010, 2011) might be responsible for the decreased MeHg concentration observed in our study over time.

4.4. THg, MeHg concentrations, carbon and nitrogen isotopes in fauna samples

Fauna inhabiting the rice paddies did show significant THg concentrations, regardless the agricultural management practices employed in the rice fields sampled. In general, among different agronomic practices, fauna in ED fields did show THg concentrations higher than EW or CW fields, although not significant differences were detected, possibly due to low statistical power (Table 3). In this regard, Sánchez-Fortún et al. (2021) in a mesocosm experiment in the same rice fields did found that common goldfish (*Carassius auratus*) exposed to ED fields also tended to accumulate higher THg concentrations than in EW or CW fields. In accordance with this, other studies have reported higher mercury concentrations in fauna samples from wetland areas associated to habitats subjected to more frequent draining events (e.g., Beau et al., 2019).

Thus, hydroperiod seems to have an influence on Hg methylation, bioavailability and accumulation in fauna inhabiting rice fields (Ackerman and Eagles-Smith, 2010; Ackerman et al., 2010; Windham-Myers et al., 2014; Sánchez-Fortún et al., 2021). Wetting-drying cycles might enhance MeHg production by transforming Hg to forms that are more easily methylated and changing redox conditions favoring Hg methylation (Marvin-DiPasquale et al., 2014), enhancing bacterial methylation and producing pulses of MeHg after re-flooding (Rothenberg and Feng, 2012; Zhao et al., 2016b; Tanner et al., 2018). Thus, since fauna mainly accumulates MeHg (Evers, 2018; Table 3) we cannot rule out the possibility that higher THg concentrations in fauna from ED fields is due to higher MeHg levels in the environment and in the food web. However, in the present study we only evaluated MeHg levels in soils and fauna for EW rice fields, and thus we cannot firmly conclude whether in our samples MeHg in soils and fauna were higher for ED fields than for those continuously flooded (EW and CW). Moreover, since Hg in fauna is acquired mostly through diet, differences in Hg concentrations in fauna from different fields managed using different agricultural practices could have been influenced by the food sources available in a particular field. Therefore, we determined $\delta^{15}N$ and $\delta^{13}C$ values to evaluate differences in diet resources for each species. Overall, similar $\delta^{15}N$ and $\delta^{13}C$ were found for the same species among field types (Table 3), nonetheless, limited samples sizes resulted in a low statistical power of the tests used to assess the differences among field types.

Finally, we evaluated the transfer of Hg through the rice field food webs, by sampling organisms from different trophic levels, as estimated from $\delta^{15}N$ signatures (Post, 2002). Regardless the agricultural management applied to the fields (CW, EW, ED) the trophic magnification factor (TMF) was > 1 for THg, indicating an effective biomagnification of Hg in all cases (Borgå et al., 2012; Conder et al., 2012). Thus, we would expect higher trophic level fauna depending on rice fields food webs in the area to be exposed to higher mercury concentrations due to biomagnification (Cotín et al., 2011; Lavoie et al., 2013; Abeysinghe et al., 2017; Du et al., 2021). Although TMF for ED was slightly higher than in EW and CW fields, it should be interpreted with caution, due to the limited sample size in the present study, and further experimental and *in-situ* studies are needed to understand mercury transfer along food webs in agricultural ecosystems and their ecological importance. When evaluating TMF for EW fields considering solely MeHg concentrations in fauna samples, the TMF value was higher than for THg, as expected, since biomagnification occurs mainly for organic mercury forms (Lavoie et al., 2013). Yet, these results reinforce the feasibility to monitor mercury concentrations in rice paddies using as bioindicators fauna species inhabiting these environments such as the crayfish (*P. clarkii*) as reported by Suárez-Serrano et al. (2010).

Overall, in this study, we demonstrated that in the Ebro Delta (NE Spain), rice fields serve as potential mercury sinks and hotspots for mercury methylation and transference to other environmental compartments (e.g., biota). Also, we evaluated how do THg concentrations in the different compartments studied (water, soil, rice plants, fauna) differ over time regarding the agricultural management employed. Interestingly, we have not found notorious differences among agricultural management techniques on THg concentrations or trends, except for fauna samples, which seemed to be affected by hydroperiod, rather than the use of fertilizers and pesticides. Finally, we have performed speciation analyses to assess the proportion of THg consisting in MeHg, the most toxic and transferrable to biota species of mercury. Unfortunately, we did not analyze MeHg levels in all the compartments studied for all the different agricultural practices employed, and thus, differences in MeHg among agricultural practices were not evaluated. Thus, further *in-situ* studies in the area are needed to evaluate whether techniques such as AWD do have an impact on organic mercury forms, mainly MeHg, in rice paddies compared to continuously flooded paddies and its trends over time on different compartments.

Synopsis

An integrative approach on rice fields' ecosystems capacity of mercury accumulation over time in soil, water, plants and fauna, with emphasis on the effect of different agricultural techniques employed for rice cultivation on mercury dynamics.

CRediT authorship contribution statement

Moisès Sánchez-Fortún: Writing – review & editing, Writing – original draft, Project administration, Investigation, Formal analysis, Conceptualization. **Josep Lluís Carrasco:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Sergi Díez:** Writing –

review & editing, Investigation, Conceptualization. **David Amouroux:** Writing – review & editing, Investigation, Formal analysis. **Emmanuel Tessier:** Writing – review & editing, Investigation. **Sophie López-Carmona:** Writing – review & editing, Investigation. **Carola Sanpera:** Writing – review & editing, Supervision, 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.118555.

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