Bian *et al.*
Food Production, Processing and Nutrition (2023) 5:63
https://doi.org/10.1186/s43014-023-00169-6

Food Production, Processing and Nutrition

RESEARCH

Open Access

Occurrence and health risks of heavy metals in crayfish (*Procambarus clarkii*) from Jiangsu province, China

Huan Bian[1], Yongzhi Zhu[1*], Limin Wen[1,2], Jingjing Ma[1], Pengpeng Li[1], Zhiming Geng[1*], Daoying Wang[1] and Weimin Xu[1]

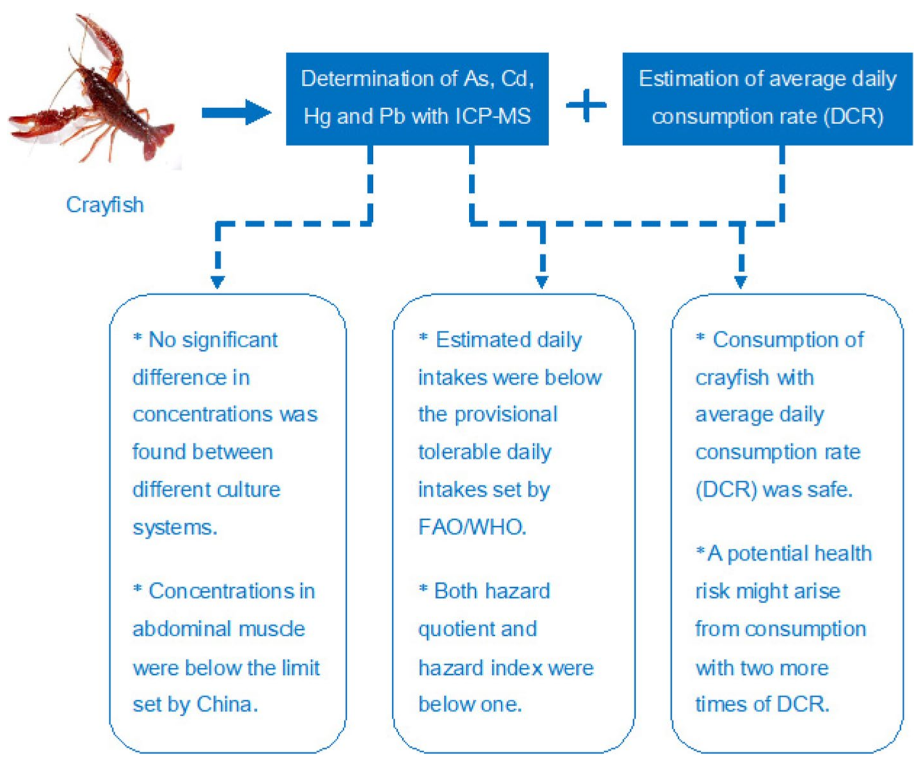
Abstract

Concentrations of arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) in crayfish from Jiangsu province, China, were measured and their health risks were evaluated. For crayfish from both crayfish-rice culture system (CRCS) and crayfish intensive culture system (CICS), concentrations of As, Cd and Pb in whole body of crayfish (WB) were significantly higher than those in abdominal muscle of crayfish (AM), while concentration of Hg in WB was significantly lower than that in AM. No significant difference in concentrations of the heavy metals was found between CRCS and CICS. Concentrations of the heavy metals in AM from both systems were below the limit set by the national standard of China. Estimated daily intake values of the heavy metals were far below the provisional tolerable daily intakes set by Joint FAO/WHO committee on Food Additives, and the corresponding hazard quotient and hazard index were below one. Therefore, consumption of crayfish with the average daily consumption rate (DCR) could be generally regarded as safe. For the consumption with two more times of average DCR during peak season, there might be a potential health risk from intakes of As and Hg in abdominal muscle of crayfish.

Keywords Crayfish, Heavy metals, Assessment of health risk, Dietary intake, Ecotoxicology

*Correspondence:
Yongzhi Zhu
yongzhizhu@163.com
Zhiming Geng
zmgeng@163.com
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Crayfish (*Procambarus clarkii*) belong to crustacean, which are native to the southern United States and the northeastern Mexico (Huner 1988). Crayfish were first introduced to Jiangsu province, China in the late of 1920s by Japanese, and gradually spread to other areas along Yangtze and Huaihe River Basins (Li et al. 2012). At present, crayfish can be found in all over the country except Tibet plateau, and are regarded as a representative invasive species (Yi et al. 2018). Crayfish had been an unpleasant existence to Chinese people, especially Chinese farmers, due to the damages of levee, dam and paddy caused by crayfish's aggressive burrowing (Jiang & Cao 2021). Such a situation stayed unchanged until 1980s, when the food value of crayfish was generally recognized and consumption of crayfish as food was favored gradually in the middle and lower reaches of Yangtze River.

At the beginning, all crayfish consumed as food was from the wild. With increasing in demand of crayfish consumption, crayfish were started to be raised in crayfish intensive culture system (CICS) and crayfish-rice co-culture system (CRCS). CICS is characterized by an efficient production of crayfish with a high stocking density and a high input of commercial feed, while CRCS effectively utilizes paddy to raise crayfish after rice harvest. Over the last twenty years, China has been the largest producer and consumer of crayfish in the world with annual output accounting for more than 90% of the world's total output (FAO 2016).

Crayfish are benthic freshwater organisms, which feed on algae, aquatic invertebrates, amphibians, fish and etc. (Gherardi 2006; Mo et al. 2022). Crayfish's omnivorous habit can accumulate hazard substances if the organisms from the lower trophic levels in the food chain are contaminated with such hazard substances as heavy metals, biotoxin, etc. (Anandkumar et al. 2020). In addition, crayfish can absorb hazard substances through gills and transport them to other organs via haemolymph (Antonín et al. 2010; El-Assal & Abdel-Meguid 2017). Crayfish survive in diverse circumstances and demonstrate an impressive tolerance to environmental stresses, and have been employed as a bio-indicator species in evaluations of chemical pollutions in water environment (Gedik et al. 2017; Samar et al. 2022a, 2022b; Suárez-Serrano et al. 2010).

Heavy metals, e.g. arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg) and etc., are classified as toxic elements without any known nutritional or beneficial effects on human health (Varol et al. 2017). On the contrary, they can exert harmful impacts even at low concentrations over a long period of time of exposure, e.g. dysfunctions of kidneys, lungs, bones, nervous system, cardiovascular system, and even development of cancers (Fang et al. 2021; Ravindra & Mor 2019). The available studies indicated that accumulation of heavy metals in crayfish was affected by the environment, culture mode and feeds, and As, Hg and etc. in crayfish might pose some potential risks to human health (Mo et al. 2022; Peng et al. 2016, 2022; Tan et al. 2021; Xiong et al. 2020). In fact, attention has always been paid to the safety and health risks arising from crayfish consumption in China. The concern about crayfish contaminated with heavy metals and/or toxins has been circulating around.

With the rapid economy development over the last decades, environmental pollution has been increasing in China. According to field surveys, the sediment and water for crayfish culturing in the middle and lower reaches of the Yangtze River Basins are contaminated with heavy metals to different degrees (Guo et al. 2020; Xiong et al. 2020; Yang et al. 2018). Jiangsu province lies in the lower reach of Yangtze River Basin, and is one of the most economically developed provinces in China. As well, Jiangsu province is the biggest producer of crayfish in the eastern coastal area, with more than $140*10^3$ hm^2 of paddy used for crayfish-rice co-culture (Jiang & Cao 2021). However, there is little of research dedicated to the crayfish in Jiangsu province, and the occurrence of heavy metals and corresponding health risks remain largely unknown. In addition, the annual consumption of crayfish has been increasing rapidly in China, from 1,110,400 tons in 2017 to 2,629,100 tons in 2021 (Yu et al. 2022), assessments available on health risk by consumption of crayfish appear to not reflect the real situation due to the ever-increasing consumption rate. Therefore, the aims of the present paper were, 1) to investigate and compare concentrations of heavy metals in crayfish from two culture systems and different districts in Jiangsu province; 2) to evaluate the potential risks arising from the consumption of crayfish.

Materials and methods

Sample collection

The study area is located in Jiangsu province, which lies in the lower reach of Yangtze River Basin with an area of 102,600 km^2 between 30°45′ ~ 35°20′ latitude and 116°18′ ~ 121°57′ longitude. Crayfish farming has spread widely over Jiangsu province, which makes it become one of the top five producers in China in terms of crayfish production. In the present work, thirty-three sampling sites distributed in seven districts, i.e. Nanjing, Zhenjiang, Lianyungang, Yancheng, Huaian, Suqian and Yangzhou (Fig. 1). They are the major producers of crayfish, and produce more than 80% of crayfish in Jiangsu province (Xu et al. 2021). Sampling was carried out in April 2022 (before rice planting). On average, twenty crayfish (each > 25 g) were caught at each of the sampling sites. All samples were immediately transported to Institute of Farm Product Processing, Jiangsu Academy of Agricultural Sciences, Nanjing, China, for further treatment and analysis.

Sample treatment and analysis

Twenty crayfish of each sampling site were randomly divided into two equal groups, i.e. groups of abdominal muscle (AM) and whole body (WB). In AM group, crayfish were dissected to obtain abdominal muscles, while in WB group the crayfish were kept intact. All samples were dried at 80 ℃ for several hours until the weight stayed unchanged. The dried samples were ground, and 0.5 g of the homogenized powder was digested with nitric acid (65%, 5 mL) in a MARS6 microwave (CEM) under a temperature gradient of 120–180 ℃ for 45 min. After cooling to room temperature, the digested samples were filtered through a cellulose acetate filter of 0.22 μm and diluted to 50 mL with water.

The concentrations of all the elements were determined using inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer NexION 350D, USA) according to "Determination of multi elements in food" (National food safety standard of China, GB 5009.268–2016). Blanks and calibration standard solutions were analyzed at a frequency of every ten samples to ensure the accuracy of analysis. The spiking recovery rates for As, Cd, Hg and Pb were 88%, 92%, 89%, and 91%, respectively. The relative standard deviation (RSD) of repeated measurements was less than 10%. The limit of detections (LOD) of As, Cd, Hg and Pb were 0.4 μg/kg, 0.4 μg/kg, 0.4 μg/kg and 2 μg/kg, while the limit of quantifications (LOQ) were 1 μg/kg, 1 μg/kg, 1 μg/kg and 5 μg/kg, respectively.

Calculation of daily consumption rate (DCR)

The DCR values for the whole year and peak season were calculated with the following Eqs. (1) and (2), respectively (Peng et al. 2016):

$$DCR_{-\text{whole year}} = \text{annual consumption} \times 0.135 \times f_1/(\text{population} \times 365) \tag{1}$$

$$DCR_{-\text{peak season}} = DCR_{-\text{whole year}} \times 365 \times f_2/120 \tag{2}$$

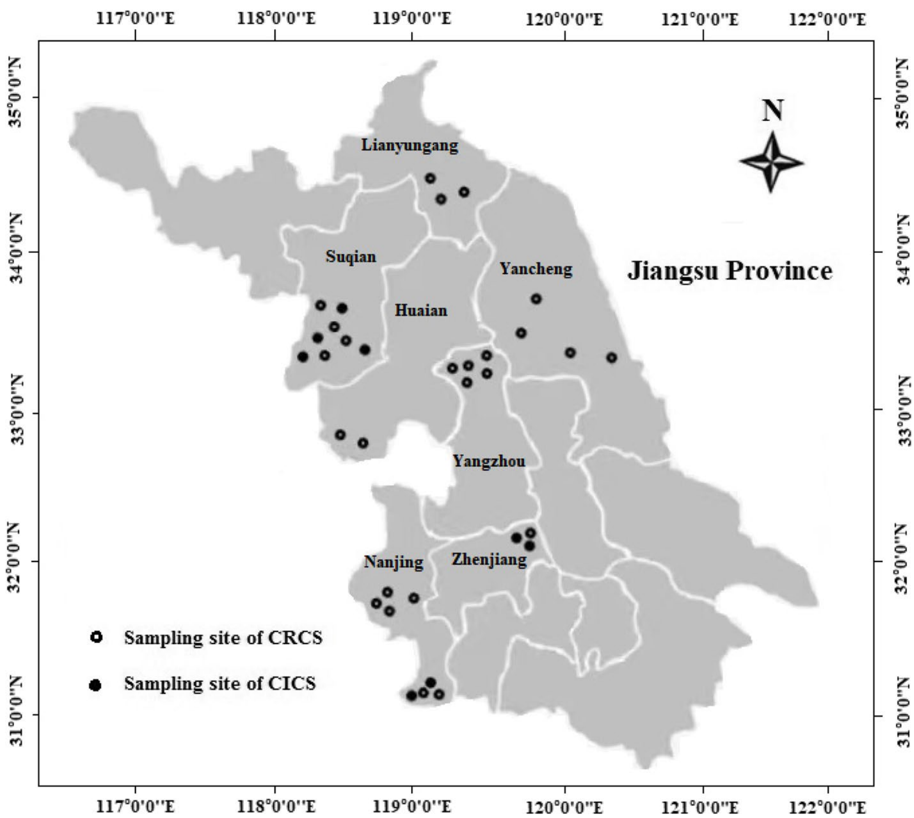


Fig. 1 Sampling sites of crayfish

Where $DCR_{\text{-whole year}}$ stands for daily consumption of crayfish per capita during a whole year in Jiangsu province (g/day/person, abdominal muscle, wet weight); annual consumption stands for consumption of crayfish per year in China, which comes from output plus import minus export; 0.135 is used to convert whole crayfish into abdominal muscle (Tan et al. 2021); f_1 is the ratio of $DCR_{\text{-whole year}}$ in Jiangsu province to that in whole country; $DCR_{\text{-peak season}}$ stands for daily consumption of crayfish per capita during the peak season (from Jun. to Sept.) in Jiangsu province (g/day, abdominal muscle, wet weight), f_2 is used to convert annual consumption to peak season consumption.

Calculation of estimated daily intake (EDI)

EDI values are used widely in risk assessment of heavy metals in food, and were calculated with the following equation (Peng et al. 2016):

$$\mathrm{EDI} = \mathrm{C} \times \mathrm{DCR}/(4.8 \times \mathrm{BW} \times 1000) \tag{3}$$

Where EDI stands for the estimated daily intake of metal through the consumption of crayfish abdominal muscle per capita (μg/kg bw/day); C is the concentration of heavy metal in crayfish abdominal muscle (μg/kg, dry weight); DCR is daily consumption of crayfish per capita (g/day, abdominal muscle, wet weight); 4.8, a conversion factor, was used to convert wet weight to dry weight according to Tan et al. (2021); BW is the body weight (kg) of an adult, and the average body weight is (60 kg) for Chinese adults (Peng et al. 2016).

Calculation of hazard quotient (HQ) and hazard index (HI)

To assess the potential risk of heavy metals' exposure due to crayfish consumption, the hazard quotient (HQ) values were calculated with the following equation:

$$\mathrm{HQ} = \mathrm{EDI}/\mathrm{RfD} \tag{4}$$

Where HQ represents hazard quotient for non-cancer health risk (unitless); EDI is the estimated daily intake (μg/kg/day), and RfD is the safe reference dose, i.e. the maximum acceptable oral dose of a heavy metal (μg/kg/day; As, 0.3; Cd, 1.0; Pb, 4.0; Hg, 0.1). It should be considered there is a potential human health hazard due to the heavy metal exposure from crayfish consumption if HQ value is bigger than one (Peng et al. 2022).

To evaluate the overall noncarcinogenic risk from multiple heavy metals, the hazard index (HI) was calculated from the following equation:

$$HI = HQ_{(As)} + HQ_{(Cd)} + HQ_{(Hg)} + HQ_{(Pb)} \quad (5)$$

HI value lower than one indicates that exposure to the four heavy metals is less likely to pose any adverse health effect, otherwise it give rise to a potential risk to human health (Peng et al. 2022).

Statistical analysis

Statistical analysis was carried out with SPSS 18.0 (IBM SPSS, USA). Heavy metal concentration below the LOQ was substituted by half the LOQ during the analysis. When the data did not meet the normality assumption, Spearman's correlation was used to test the correlation hypotheses and a nonparametric test was used for group comparison.

Results and discussion

Concentrations of heavy metals in crayfish

The maximum, minimum, average, standard deviation and detection frequency of concentration of the four metals in crayfish were listed in Table 1. In CRCS, the average concentrations of As, Cd, Hg and Pb in whole body of crayfish (WB) were 1871.7 μg/kg, 103.01 μg/kg, 54.59 μg/kg and 354.58 μg/kg, respectively, while those in abdominal muscles of crayfish (AM) were 563.85 μg/kg, 4.92 μg/kg, 136.55 μg/kg and 54.41 μg/kg, respectively. As for CICS, the average concentrations of As, Cd, Hg and Pb in WB were 1676.54 μg/kg, 270.88 μg/kg, 53.29 μg/kg and 27.04 μg/kg, respectively, while those in AM were 634.59 μg/kg, ND, 130.58 μg/kg and 19.81 μg/kg, respectively. Four metals were detected in all the samples with detection frequency (DF) of 100%, except that Cd was detected in AM samples from CRCS and CICS with DF of 24% and 0%, respectively, and Pb was detected in AM samples from CRCS and CICS with DF of 80% and 50%, respectively. Concentrations of the four metals in AM from both culture systems were all below the limit of 2400 μg/kg (dry weight), converted from 500 μg/kg (wet weight) set by China's national standard (GB2762-2017), in which a factor of 4.8 was used to convert "wet weight" to "dry weight" (Tan et al. 2021). As for WB, Cd, Hg and Pb were found to be lower than the limit, while five out of thirty-three samples were found to contain higher concentration of As than 2400 μg/kg. Considering that abdominal muscle of crayfish is the target part for human consumption, all the crayfish in the present study could be regarded as safe for food.

Relationships among heavy metals in crayfish

For the four heavy metals, there was no significant difference in concentrations between culture systems, no matter whether in AM or in BW. As displayed in Fig. 2, significant lower concentrations of As, Cd and Pb, and significant higher concentration of Hg were found in AM than WB ($p < 0.01$) for both culture systems. Crayfish accumulate heavy metals by feeding on the polluted organisms from the lower trophic levels in the food chain and absorbing of gills from the ambient water. The bioenrichment of heavy metals in crayfish is a time- and dose-dependent process, with different distributions in tissues for different heavy metals. Cd, Pb and etc. are thought to be accumulated mainly in hepatopancreas, gills and exoskeleton, while Hg is more inclined to be enriched in muscle (Antonín et al. 2010). Due to such

Table 1 Concentrations of heavy metals in crayfish from CRCS and CICS in Jiangsu province, China (μg/kg, dry weight)*

Source	As		Cd		Hg		Pb	
	AM	WB	AM	WB	AM	WB	AM	WB
CRCS								
Min	265.20	834.86	ND	44.21	60.43	17.71	ND	132.34
Max	1049.76	4379.81	25.49	233.52	293.00	91.68	224.54	949.78
Mean	563.85 a	1871.78 b	4.92 a	103.01 b	136.55 b	54.59 a	50.41 a	354.58 b
SD	231.93	866.67	8.35	50.91	61.72	23.83	46.26	192.31
DF	100	100	24	100	100	100	80	100
CICS								
Min	415.10	717.98	ND	48.86	74.88	31.49	ND	121.97
Max	1085.33	2846.83	ND	740.30	167.38	98.88	58.66	584.88
Mean	634.59 a	1676.54 b	ND a	270.88 b	130.58 b	53.29 a	19.81 a	270.04 b
SD	226.86	669.61	/	229.43	34.11	24.45	21.12	153.46
DF	100	100	0	100	100	100	50	100

LOD of As, Cd, Hg and Pb were 0.4 μg/kg, 0.4 μg/kg, 0.4 μg/kg and 2 μg/kg; Different lower-case letters on columns for the same heavy metal are significantly different ($p < 0.01$)

* *AM* Abdominal muscle, *WB* Whole body, *ND* Not detected, *SD* Standard deviation, *DF, %* Detection frequency

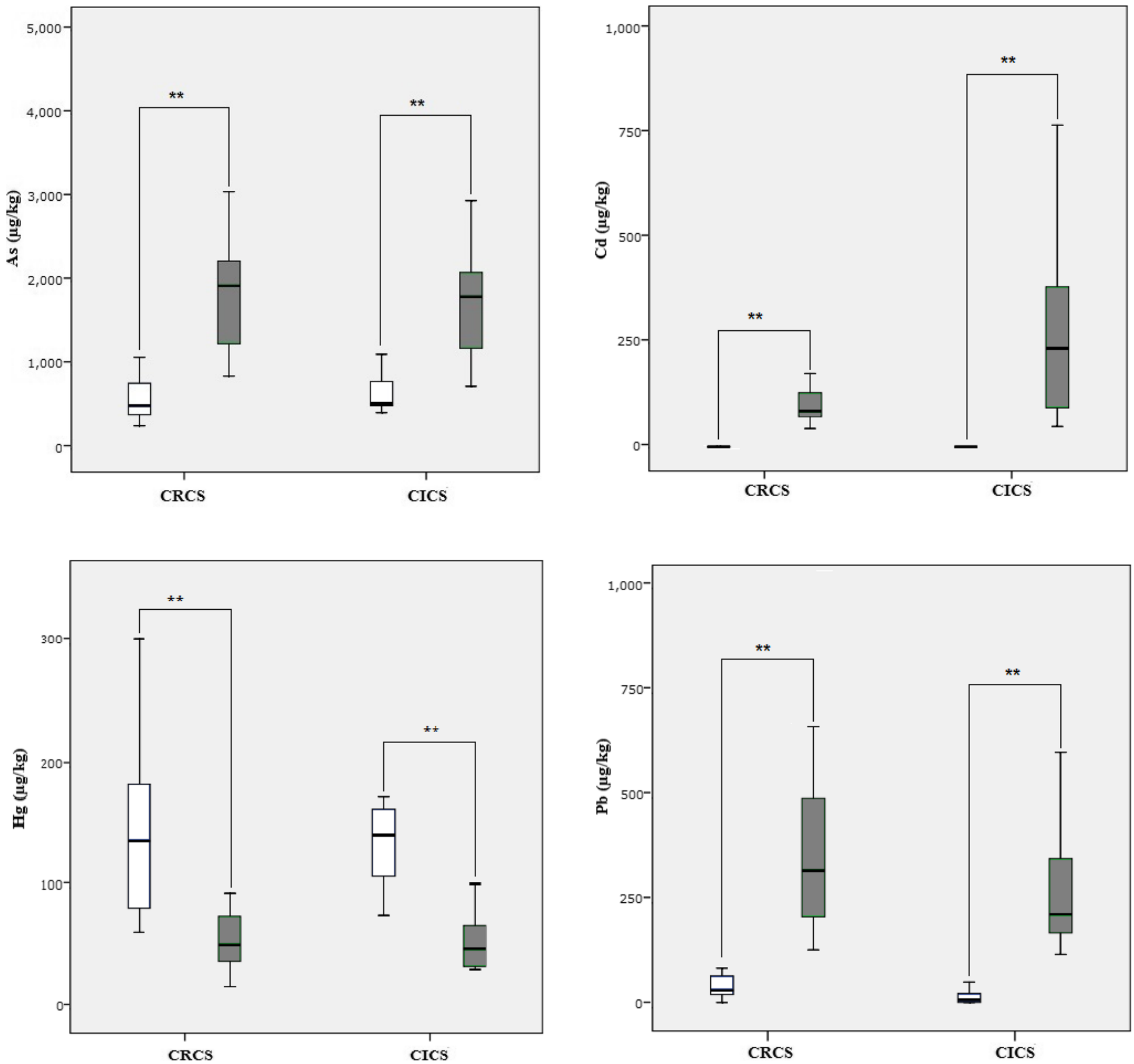


Fig. 2 Concentrations of As, Cd, Hg and Pb in AM (white box) and WB (grey box) of crayfish from CRCS and CICS (µg/kg, dry weight). The line in the box represents the median value; the bottom and the top of each box represent 5th and 95th percentiles, respectively; ** represents $p < 0.01$. Heavy metal concentration below the LOQ was substituted by half the LOQ during the analysis. LOQ of As, Cd, Hg and Pb were 1 µg/kg, 1 µg/kg, 1 µg/kg and 5 µg/kg, respectively

accumulation characteristics of heavy metals in crayfish, in CRCS of the present study, average concentrations of As, Cd and Pb were 3.32 times, 20.94 times and 7.03 times higher in WB than those in AM, respectively, while average concentration of Hg was 2.50 times higher in AM than that in WB. The same phenomenon was also found in CICS. Such accumulation patterns of heavy metals in crayfish, i.e. lower levels of As, Cd and Pb with a higher level of Hg in AM than in WB, was in consistent with previous reports (Alcorlo et al. 2006; Gedik et al. 2017; Anandkumar et al. 2020; Peng, et al. 2022). Hg in crayfish is determined as total mercury by ICP-MS method, and includes inorganic mercury and methyl mercury. In fact, inorganic mercury is accumulated in crayfish in the similar way as Cd, Pb and etc., i.e. hepatopancreas > gills > exoskeleton > abdominal muscle, while the accumulation of methyl mercury is in a different pattern: gills > abdominal muscle > hepatopancreas > exoskeleton (Wright et al. 1991). Methyl mercury is found to be accumulated in proteins due to its binding to the sulphur-containing amino acid cysteine (Lemes & Wang 2009). Inorganic mercury can be transformed into methyl mercury through methylation by sulphate-reducing bacteria in sediments (Parks et al. 2013), and methyl mercury has been reported to represent approximately 90% of the total mercury in crayfish (Antonín et al. 2010). Therefore, the higher concentration of Hg in abdominal muscles could be attributed to the relatively high protein content in abdominal muscles and a higher level of methyl mercury in environment.

In the present study, concentrations of the heavy metals in AM from the two culture systems were in the order of As > Hg > Cd > Pb with significant differences among them ($p < 0.01$). As for WB, the order was As > Pb > Cd > Hg with significant differences among them ($p < 0.01$) for CRCS, while it was As > Pb ≈ Cd > Hg with significant differences among them ($p < 0.01$), except between Pb and Cd, for CICS.

In CRCS, concentrations of As and Hg in AM were found to be significantly and positively correlated with concentrations of As (0.600, $p < 0.01$) and Hg (0.680, $p < 0.01$) in WB, respectively; and concentration of As in WB was found to be significantly and positively correlated with concentration of Hg (0.432, $p < 0.05$) in WB. As for CICS, there was no significant correlation between concentrations of heavy metals in AM and WB, and among concentrations of heavy metals in AM (or WB) either. Crayfish accumulate heavy metals through foraging and breathing, and heavy metals may come from sediments, fertilizers, feeds and etc. In CRCS, significantly positive correlation for concentration of As and Hg between in AM and WB indicated that As and Hg were accumulated in AM in a similar way to their accumulation in WB. Significantly positive correlations between concentrations of As and Hg in WB indicated that the two metals might come from the same pollution source. Compared with CRCS, more commercial feeds being used in CICS (Bosma & Verdegem 2011; Li et al. 2019), which resulted in more disturbances to concentrations of heavy metals in ambient water, might be the major cause of no significant correlation between/among heavy metals in crayfish from CICS.

Differences of heavy metals in crayfish among districts

In order to investigate the differences of heavy metals in crayfish among districts, concentrations of heavy metals in AM and WB were collected for different districts without differentiation of two culture systems, and a nonparametric test was carried out for group comparison. There was no significant difference in concentrations of heavy metals among districts, except that the concentration of Cd in AM from Lianyungang was found to be significantly higher than those from other districts ($p < 0.05$), i.e. Suqian, Nanjing, Yangzhou, Zhenjiang and Huaian (Figs. 3 and 4). Although there lacked statistically significant differences among districts, the highest levels of As, Hg and Pb in AM (from Lianyungang, Huaian and Huaian, respectively) were found to be 2.6, 2.2 and 2.3 times higher than the corresponding lowest ones (from Huaian, Yancheng and Zhenjiang, respectively), respectively. The similar situation was found in WB from different districts.

Table 2 listed concentrations of heavy metals in abdominal muscle of crayfish in the present study and other publications. The contamination of heavy metals in crayfish abdominal muscle in the present study was similar

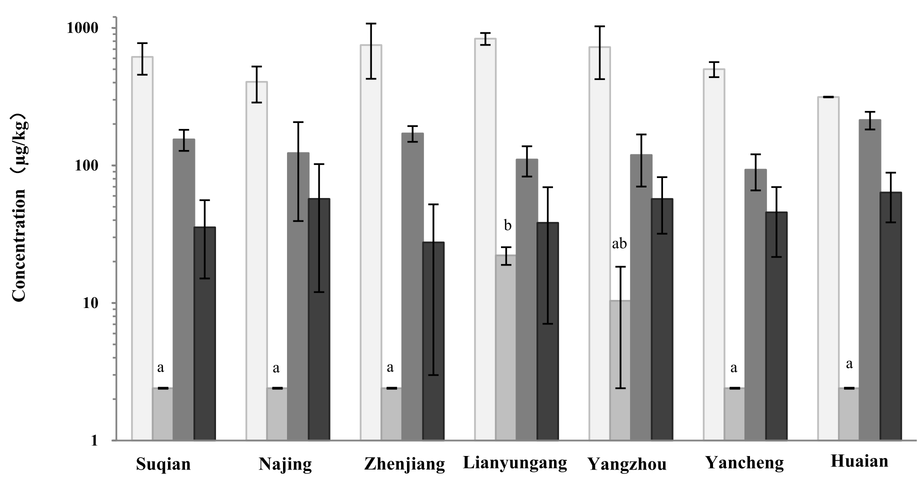


Fig. 3 Concentrations of heavy metals in abdominal muscle of crayfish from different districts. -As, -Cd, -Hg, -Pb. Different lower-case letters on columns for the same heavy metal are significantly different ($p < 0.05$). Heavy metal concentration below the LOQ was substituted by half the LOQ during the analysis. LOQ of As, Cd, Hg and Pb were 1 μg/kg, 1 μg/kg, 1 μg/kg and 5 μg/kg, respectively

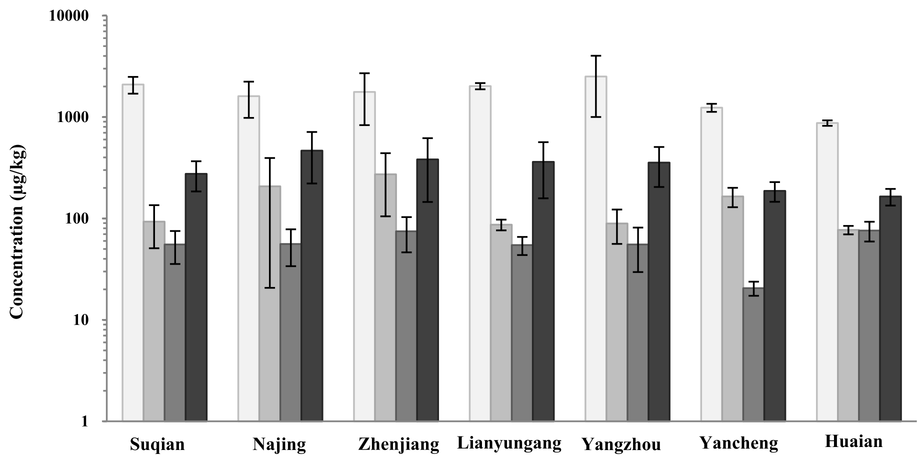


Fig. 4 Concentrations of heavy metals in whole body of crayfish from different districts. -As, -Cd, -Hg, -Pb. Heavy metal concentration below the LOQ was substituted by half the LOQ during the analysis. LOQ of As, Cd, Hg and Pb were 1 μg/kg, 1 μg/kg, 1 μg/kg and 5 μg/kg, respectively

Table 2 Concentrations of heavy metals in abdominal muscle of crayfish in the present study and other publications (mg/kg, dry weight)[a]

Location	As	Cd	Hg	Pb	Ref
Jiangsu, China					
CRCS	0.27 ~ 1.05	ND ~ 0.025	0.060 ~ 0.29	ND ~ 0.22	Present study
CICS	0.42 ~ 1.09	ND	0.075 ~ 0.17	ND ~ 0.059	
Jiangsu, China	1.13	0.024	0.22	0.24	Anandkumar et al. 2020
Hubei, China (wet weight)					
wild	0.14	1.123	-	1.88	Xiong et al. 2020
culture	0.11	0.47	-	1.33	
Hunan/Hubei, China	0.56	0.0046	-	0.13	Tan et al. 2021
Louisiana, USA	0.2 ~ 3.7	0.06	-	4.5	Gedik et al. 2017
South-Western Sicily, Italy	1.8	0.0	-	0.2	Bellante et al. 2015
River Nile, Egypt	-	0.05	0.47	0.32	Farrag et al. 2022

LOD of As, Cd, Hg and Pb were 0.4 μg/kg, 0.4 μg/kg, 0.4 μg/kg and 2 μg/kg, respectively

[a] *ND* Not detected

to the result reported by Anandkumar et al. (2020), who surveyed the heavy metals in crayfish from local aquatic product markets in Zhenjiang, a city in Jiangsu province. However, it was fairly common that there were huge differences among contaminations of heavy metals in crayfish abdominal muscle from different papers. For example, Peng et al. (2022) and Xiong et al. (2020) studied the crayfish from Hubei province, the top producer of crayfish in China, respectively, and gave totally different contamination patterns of heavy metals. Rodríguez-Estival et al. (2019) found Hg and Pb in crayfish abdominal muscles from polluted environments (mining districts) could be 37 times and 175 times than those from normal environments, respectively. Therefore, such differences in contaminations of heavy metals should be related to differences in pollution of sampling sites (Farrag, et al. 2022). Since the growth cycle of crayfish is roughly the same in culture systems, pollution level of heavy metals in environment and in commercial feeds can be regarded as the determinant factors for contamination of heavy metals in crayfish.

DCR for people in Jiangsu province, China

Among the assessments of health risk of heavy metals in crayfish available, there are huge differences in EDI and HQ values, which could be primarily ascribed to the differences among estimations of DCR and concentrations of heavy metals. Unlike concentrations of heavy metals, which are determined precisely with the method based on ICP-MS, DCR can only be estimated by researchers on the basis of different data. For examples, DCR values of 168 g and 55 g crayfish abdominal muscle were suggested for adults in Hubei province, the top producer of crayfish in China, by Peng et al. (2022) and Xiong et al. (2020), respectively. The former was three times higher than the later. In the present study, in order to reflect the real consumption of crayfish as much as possible in Jiangsu province, DCR was estimated based on the following respects: 1) daily consumption of crayfish per capita in China; 2) the difference between daily consumption of crayfish per capita in China and in Jiangsu province; 3) the difference between daily consumption of crayfish per capita during a whole year and peak season (Jun.-Sept.).

In 2021, total of 2,629,100 tons of crayfish was consumed (Yu et al. 2022), by 1412.6 million people in China (NBSC (China's National Bureau of Statistics) 2021), which meant national average daily consumption of crayfish per capita was 5.1 g of crayfish. There are huge differences in popularity of crayfish consumption among different regions in China. In Nanjing, the capital city of Jiangsu province, the daily consumption of crayfish per capita could be 27 times higher than the national average value (Peng et al. 2016). In the present paper, a conservative value of 20, i.e. the "f_1" in Eq. (1), was used to estimate the daily consumption of crayfish per capita in Jiangsu province based on the national average value. Considering more than 90% of annual consumption of crayfish occurred during peak season from Jun. to Sept. (Peng et al. 2016), 0.9, i.e. the "f_2" in Eq. (2), was used to estimate the daily consumption of crayfish per capita during peak season in Jiangsu province. Therefore, in the present study, $DCR_{\text{-whole year}}$ and $DCR_{\text{-peak season}}$ were found to be 13.8 (g/day/person, abdominal muscle, wet weight) and 37.7 (g/day/person, abdominal muscle, wet weight), respectively.

Table 3 EDI, HQ and HI of heavy metals by consuming abdominal muscle of crayfish for whole year and peak season[a]

	As	Cd	Hg	Pb
CRCS				
$EDI_{\text{-whole year}}$	0.027	0.000	0.007	0.002
$EDI_{\text{-peak season}}$	0.074	0.001	0.018	0.007
$HQ_{\text{-whole year}}$	0.091	0.000	0.065	0.001
$HQ_{\text{-peak season}}$	0.246	0.001	0.179	0.002
$HI_{\text{-whole year}}$	0.157			
$HI_{\text{-peak season}}$	0.428			
CICS				
$EDI_{\text{-whole year}}$	0.030	0.000	0.006	0.001
$EDI_{\text{-peak season}}$	0.083	0.000	0.017	0.003
$HQ_{\text{-whole year}}$	0.101	0.000	0.063	0.000
$HQ_{\text{-peak season}}$	0.277	0.000	0.171	0.001
$HI_{\text{-whole year}}$	0.164			
$HI_{\text{-peak season}}$	0.448			

[a] EDI, μg/kg bw/day

Human health risk assessment

Based on the concentrations of heavy metals in abdominal muscles of crayfish and DCR estimated for adults, corresponding values of EDI, HQ and HI were calculated and listed in Table 3. All $EDI_{\text{-peak season}}$ values were remarkably higher than corresponding $EDI_{\text{-whole year}}$ values, due to the higher DCR of peak season (from Jun. to Sept.) compared with that of whole year, except for the EDI values of Cd of crayfish from CICS. Accordingly, the similar situation was occurred for values of HQ and HI.

EDI is used to evaluate the daily exposure to a pollutant through diets. In the present study, EDI values of heavy metals, whether for whole year or for peak season, were far below the provisional tolerable daily intake (PTDI) suggested by Joint FAO/WHO committee on Food Additives (Anandkumar et al. 2020), which are 2.14, 1.00, 0.23 and 3.57 (μg/kg bw/day) for As, Cd, Hg and Pb, respectively. Therefore, intakes of heavy metals from consumption of crayfish in the present paper would not pose any health risk to consumers.

HQ is commonly employed to evaluate the non-carcinogenic risk level due to lifetime exposure to a pollutant, and is calculated by comparing EDI with RfD, the reference (safe) oral dose of the pollutant (Anandkumar et al. 2020). HQ below one indicates the exposure to the pollutant poses no adverse impact on human health (Peng et al. 2022). In the present study, for CRCS, $HQ_{\text{-whole year}}$ values of As and Hg were 0.091 and 0.065, respectively, while their $HQ_{\text{-peak season}}$ values were 0.246 and 0.179, respectively. Almost the same levels of HQ of As and Hg were found for CICS. For both culture systems, HQ values of Cd and Pb were found to be close to zero. Therefore, consumption of crayfish from the seven districts in Jiangsu province, China, could be generally regarded as safe based on the average consumption rates of whole year and peak season, and there was no significant health risk from intake of individual heavy metals in abdominal muscle of crayfish.

HI is used to evaluate the overall non-carcinogenic risk due to exposure to multiple pollutants, and is expressed by the sum of HQ values of the pollutants involved (Peng et al. 2022). In the present paper, values of $HI_{\text{-whole year}}$ and $HI_{\text{-peak season}}$ were 0.157 and 0.428 for CRCS, and 0.164 and 0.448 for CICS, respectively. HQ values of As and Hg accounted for more than 99% of HI in all cases. HI values were below one, whether for whole year or for peak season, indicating that the exposure to the four heavy metals through consumption of crayfish abdominal muscle would not result in overall non-carcinogenic risk to human health either.

However, it was worthy of note that there are huge differences in popularity of crayfish eating among people of different ages, just as the case among different regions in China. Young people are found to be the major consumer of crayfish with the highest consumption rate (Bai et al. 2019; Peng et al. 2016), the $DCR_{\text{-peak season}}$ value for young people may be much higher than the $DCR_{\text{-peak season}}$ value calculated for average residents. In the present paper, when $DCR_{\text{-peak season}}$ value reached two times higher than the average $DCR_{\text{-peak season}}$ value, i.e. 75.4 (g/day/person, abdominal muscle, wet weight), $HI_{\text{-peak season}}$ values would be 0.856 and 0.896 for CRCS and CICS, respectively. When $DCR_{\text{-peak season}}$ value reached two and a half times higher than the average $DCR_{\text{-peak season}}$ value, i.e. 94.25 (g/day/person, abdominal muscle, wet weight), $HI_{\text{-peak season}}$ values would be 1.07 and 1.12 for CRCS and CICS, respectively. In fact, some researchers suggested the DCR value might be as much as 135 (g/day/person, abdominal muscle, wet weight, Xu et al. 2022) and 168 (g/day/person, abdominal muscle, wet weight, Peng et al. 2022). Therefore, consumption of crayfish during peak season might pose a potential health risk to those consumers (e.g. young people) with two more times of the average $DCR_{\text{-peak season}}$ value. Such a potential health risk was mainly resulted from intakes of As and Cd by consuming abdominal muscle of crayfish.

Conclusion

There was no significant difference in concentrations of the four heavy metals in crayfish between CRCS and CICS, and no significant difference in concentrations of heavy metals was found among districts except for the concentration of Cd in AM from Lianyungang being significantly higher than those from other districts. WB was found to accumulate higher concentrations of As, Cd and Pb, and a lower concentration of Hg than AM. Concentrations of the four heavy metals in AM of crayfish were below the limit set by the national standard of China.

EDI values of the four heavy metals were far below PTDI suggested by Joint FAO/WHO committee on Food Additives. HQ and HI values by consuming abdominal muscle of crayfish were below one. Consumption of crayfish from the seven districts in Jiangsu province, China, could be generally regarded as safe. However, for those young consumers with higher DCR values (e.g. two more times of the average DCR) during peak season, there might be a potential health risk. Such a potential health risk was mainly from intakes of As and Cd by consuming abdominal muscle of crayfish.

Acknowledgements
The authors would like to thank Dr. Fang Liu and Dr. Chong Sun for suggestions on the research proposal and data interpretation.

Authors' contributions
H. Bian and L. Wen - conceptualization and project administration; J. Ma and P. Li - assisting part of the experiments and data analysis; Y. Zhu and D. Wang - funding acquisition and methodology; Z. Geng and W. Xu - supervision and manuscript-writing. All authors have read and agreed to the published version of the manuscript.

Funding
This study was financially supported by the earmarked fund for Jiangsu Modern Agricultural Industry (Red Swamp Crayfish) Technology System [JATS (2022) 444], and Jiangsu Agricultural Science and Technology Innovation Fund [CX (21) 2030].

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
Dr. Daoying Wang is a member of Editorial Board of ***Food Production, Processing and Nutrition*** and he was not involved in the journal's review of, or decisions related to this manuscript.

Author details
[1]Institute of Farm Product Processing, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, P. R. China. [2]College of Food Science, Tibet Agricultural & Animal Husbandry University, Linzhi 860000, P. R. China.

Received: 24 October 2022 Accepted: 11 May 2023
Published online: 03 December 2023

References

Alcorlo, P., Otero, M., Crehuet, M., Baltanás, A., & Montes, C. (2006). The use of the red swamp crayfish (Procambarus clarkii, Girard) as indicator of the bioavailability of heavy metals in environmental monitoring in the River Guadiamar (SW, Spain). *Science of the Total Environment, 366*, 380–390.
Anandkumar, A., Li, J., Prabakaran, K., Jia, Z. X., Leng, Z. R., Nagarajan, R., & Du, D. L. (2020). Accumulation of toxic elements in an invasive crayfish species (Procambarus clarkii) and its health risk assessment to humans. *Journal of Food Composition and Analysis, 88*, 103449.
Antonín, K., Mološ, B., & Pavel, K. (2010). Bioaccumulation and effects of heavy metals in crayfish: A review. *Water, Air and Soil Pollution, 211*, 5–16.
Bai, L., Xu, M. J., Li, W. W., Han, H. H., Liu, J. K., Fu, K. P., & Guo, Y. C. (2019). Retrospective case analysis of crayfish-transmitted Haff disease in China during 2016–2017. *Food Control, 104*, 181–186.
Bellante, A., Maccarone, V., Buscaino, G., Buffa, G., Filiciotto, F., Traina, A., Del Core, M., & …Sprovieri, M. (2015). Trace element concentrations in red swamp (Procambarus clarkii) and surface sediments in Lake Preola and Gorghi Tondi natural reserve, SW Sicily. *Environmental Monitoring and Assessment, 187*, 1–18.
Bosma, R. H., & Verdegem, M. C. J. (2011). Sustainable aquaculture in ponds: Principles, practices and limits. *Livestock Science, 139*, 58–68.
El-Assal, F. M., & Abdel-Meguid, Z. A. (2017). Impact of heavy metal pollution on Procambarus clarkii (Crustacea: Decapoda), from Egypt. *International Journal of Waste Resources, 7*, 1–13.
Fang, H. X., Gui, H. R., Li, J., Yu, H., Wang, M. C., Jiang, Y. Q., & Huang, Y. H. (2021). Risks assessment associated with different sources of metals in

abandoned soil of zhuxianzhuang coal mine, Huaibei Coalfield (Anhui, China). *Bulletin of Environmental Contamination and Toxicology, 106*, 370–376.
FAO. (2016). *Fisheries and aquaculture resources. Cultured aquatic species information programme Procambarus clarkii (Girard, 1852)*. http://www.fao.org/fishery/culturedspecies/Procambarus_clarkii/en.
Farrag, M. M. S., El-Geddawy, M.-A.M.A., & Ahmed, Z. S. A. (2022). More evidences for the nutritional quality and future exploitation of the invasive crayfish Procambarus clarkii (Girard, 1852) from the River Nile, Egypt. *Egyptian Journal of Aquatic Research, 48*, 151–156.
Gedik, K., Kongchum, M., DeLaune, R. D., & Sonnier, J. J. (2017). Distribution of arsenic and other metals in crayfish tissues (Procambarus clarkii) under different production practices. *Science of the Total Environment, 574*, 322–331.
Gherardi, F. (2006). Crayfish invading Europe: The case study of Procambarus clarkii. *Marine and Freshwater Behavior and Physiology, 39*, 175–191.
Guo, B., Hong, C. L., Tong, W. B., Xu, M. X., Huang, C. L., Yin, H. Q., & Fu, Q. L. (2020). Health risk assessment of heavy metal pollution in a soil-rice system: a case study in the Jin-Qu Basin of China. *Scientific Reports, 10*, 11490.
Huner, J. V. (1988). Status of crayfish transplantations. *Freshwater Crayfish, 7*, 29–34.
Jiang, Y., & Cao, C. G. (2021). Crayfish-rice integrated system of production: an agriculture success story in China. *Agronomy for Sustainable Development, 41*, 68.
Lemes, M., & Wang, F. (2009). Methylmercury speciation in fish muscle by HPLC-ICP-MS following enzymatic hydrolysis. *Journal of Analytical Atomic Spectrometry, 24*, 663–668.
Li, F. B., Feng, J. F., Zhou, X. Y., Xu, C. C., Jijakli, M. H., Zhang, W. J., & Fang, F. P. (2019). Impact of rice-fish/shrimp co-culture on the N2O emission and NH3 volatilization in intensive aquaculture ponds. *Science of the Total Environment, 655*, 284–291.
Li, Y. H., Guo, X. W., Cao, X. J., Deng, W., Luo, W., & Wang, W. M. (2012). Population genetic structure and post-establishment dispersal patterns of the red swamp crayfish Procambarus clarkii in China. *PLoS ONE, 7*, e40652.
Mo, A. J., Dang, Y., Wang, J. H., Liu, C. S., Yang, H. J., Zhai, Y. X., & Yuan, Y. C. (2022). Heavy metal residues, releases and food health risks between the two main crayfish culturing models: Rice-crayfish co-culture system versus crayfish intensive culture system. *Environmental Pollution, 305*, 119216.
NBSC (China's National Bureau of Statistics). (2021). *China economic annual report*.
Parks, J. M., Johs, A., Podar, M., Bridou, R., Hurt, R. A., Smith, S. D., & Liang, L. Y. (2013). The genetic basis for bacterial mercury methylation. *Science, 339*, 1332–1335.
Peng, Q., Greenfield, B., Dang, F., & Zhong, H. (2016). Human exposure to methylmercury from crayfish (Procambarus clarkii) in China. *Environmental Geochemistry and Health, 38*, 169–181.
Peng, F. J., Li, J. W., Gong, Z. Y., Yue, B., Wang, X. L., Manyande, A., & Du, H. Y. (2022). Investigation of bioaccumulation and human health risk assessment of heavy metals in crayfish (Procambarus clarkii) farming with a rice-crayfish-Based co-culture breeding modes. *Foods, 11*, 261.
Ravindra, K., & Mor, S. (2019). Distribution and health risk assessment of arsenic and selected heavy metals in Groundwater of Chandigarh, India. *Environmental Pollution, 250*, 820–830.
Rodríguez-Estival, J., Morales-Machuca, C., Pareja-Carrera, J., Ortiz-Santaliestra, M. E., & Mateo, R. (2019). Food safety risk assessment of metal pollution in crayfish from two historical mining areas: Accounting for bioavailability and cooking extractability. *Ecotoxicology and Environmental Safety, 185*, 109682.
Samar, M., Ayman, S. M., Haggag, A. M., & Wafaa, S. F. (2022). The Effect of zinc zoncentration on physiological, immunological, and histological changes in crayfish *(Procambarus clarkii)* as bio-indicator for environment quality criteria. *Biological Trace Element Research, 200*, 375–384.
Samar, M., Ayman, S. M., Haggag, A. M., & Wafaa, S. F. (2022). Zn contamination stimulate agonistic behavior and oxidative stress of crayfishes (*Procambarus clarkii*). *Journal of Trace Elements in Medicine and Biology, 69*, 126895.
Suárez-Serrano, A., Alcaraz, C., Ibáñez, C., Trobajo, R., & Barata, C. (2010). Procambarus clarkii as a bioindicator of heavy metal pollution sources in the lower Ebro River and Delta. *Ecotoxicology and Environmental Safety, 73*, 280–286.
Tan, Y. Y., Peng, B., Wu, Y. L., Xiong, L. J., Sun, J. X., Peng, G. H., & Bai, X. F. (2021). Human health risk assessment of toxic heavy metal and metalloid intake via consumption of red swamp crayfish (Procambarus clarkii) from rice-crayfish co-culture fields in China. *Food Control, 128*, 108181.
Varol, M., Kaya, G. K., & Alp, A. (2017). Heavy metal and arsenic concentrations in rainbow trout (Oncorhynchusmykiss) farmed in a dam reservoir on the Firat (Euphrates) River: Risk-based consumption advisories. *Science of the Total Environment, 599–600*, 1288–1296.
Wright, D. A., Welbourn, P. M., & Martin, A. V. M. (1991). Inorganic and organic mercury uptake and loss by the crayfish Orconectes propinquus. *Water, Air, and Soil Pollution, 56*, 697–707.
Xiong, B., Xu, T., Li, R. P., Johnson, D., Ren, D., Liu, H. G., & Huang, Y. P. (2020). Heavy metal accumulation and health risk assessment of crayfish collected from cultivated and uncultivated ponds in the Middle Reach of Yangtze River. *Science of the Total Environment, 739*, 139963.
Xu, Y., Xu, Z. Q., Huang, H. B., Yan, W. H., Tang, J. Q., Xia, A. J., & Pan, J. L. (2021). Development of crayfish industry in Jiangsu. *Aquiculture, 10*, 77–80.
Xu, J. Y., Zhu, Z., Zhong, B. S., Gong, W. R., Du, S. J., Zhang, D., & Lu, S. Y. (2022). Health risk assessment of perchlorate and chlorate in red swamp crayfish (Procambarus clarkii) in China. *Science of the Total Environment, 842*, 156889.
Yang, Q. Q., Li, Z. Y., Lu, X. N., Duan, Q. N., Huang, L., & Bi, J. (2018). A review of soil heavy metal pollution from industrial and agricultural regions in China: Pollution and risk assessment. *Science of the Total Environment, 642*, 690–700.
Yi, S. K., Li, Y. H., Shi, L. L., Zhang, L., Li, Q. B., & Chen, J. (2018). Characterization of population genetic structure of red swamp crayfish, Procambarus clarkii, in China. *Scientific Reports, 8*, 5586.
Yu, X. J., Hao, X. J., Dang, Z. Q., Yang, L. K., Wang, X. G., Zhang, Y. H., & Zhang, Y. (2022). Report on the development of crawfish industry in China (2022). *China Fisheries, 6*, 47–54.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.