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Assessment of the nutritional profiles and potentially toxic elements of wild and farmed freshwater fish in Cambodia

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ABSTRACT

Climate change and pollution are threatening inland freshwater ecosystems which contribute to human well-being by providing food and incomes. To address this issue, aquaculture is expanding sometimes in intensive settings. We aimed to assess the nutritional and contaminant profiles of three fish species (*Channa micropeltes*, *Pangasianodon hypophthalmus*, *Clarias microcephalus*) from the wild ecosystem and caged culture in the Tonle Sap Lake. The mineral profile was assessed by ICP-MS and the overall nutritional quality was characterized by SAIN-LIM scores. Using data on fish consumption by pregnant women, we estimated the daily intakes of several potentially toxic elements. Overall, fish species had similar nutritional profiles, regardless of production method. Only lipid content was higher in caged systems. The production method had no influence on mineral profile and potentially toxic element contents except for mercury higher among wild *Pangasianodon hypophthalmus* (2.1 µg/100 g). With a consumption of 108 g of fish per day per woman, the median estimated daily intakes of potentially toxic elements were below the tolerable daily limits. However, three women who ate large quantities of fish had mercury intakes (30–32 µg/day) that exceeded the tolerable daily intake. When the rice consumption was taken into account, a high number of women had inorganic arsenic intakes (20–80 µg/day) exceeding the tolerable daily limits. This work is a contribution to the assessment of the risks of arsenic and mercury exposure for pregnant women in Cambodia integrating real food consumption data.

1. Introduction

Over the last few decades, the fishing and aquaculture sectors have been increasingly recognized for their essential contribution to the development of human societies. They generate income and employment, and clearly contribute to food security. Some 58.5 million people depend on these sectors for their employment in developing countries (FAO, 2022). With population growth and rising incomes in Asia and Africa, the demand for fish is constantly increasing.

Cambodia ranks seventh in terms of fish caught in inland waters (FAO, 2022). Annual production reaches 0.41 million tonnes, 75 % of which comes from the inland Tonle Sap Lake (TSL), the largest freshwater lake in South-East Asia. Fishery makes a remarkable contribution to the country's economic growth, estimated at 10 % of gross domestic product (Yoshimura et al., 2022). The fish caught come either from the wild ecosystem or aquaculture production in cages, ponds and pens. Cage farming accounts for around 53 % of total production and 77 % of these farms are located in the TSL (Puy, 2022).

Urban growth, poor waste management by communities or cities, agricultural or industrial residues all lead to water pollution, particularly of inland freshwater. The TSL ecosystem is threatened not only by this pollution, but also by illegal fishing and the growing number of dams built upstream on the Mekong (Bao et al., 2022). In addition, more than two million people live in floating villages in TSL with poor waste management. Multi-drug-resistant bacteria, pesticide residues and potentially toxic elements were identified in this area (Yoshimura et al., 2022). The TSL was reported to be polluted by domestic waste from floating villages, agricultural activities, urban waste, and gold mining (Soum et al., 2021). Potentially toxic residues such as mercury, cadmium, lead, arsenic or pesticides (dichlorodiphenyltrichloroethane,

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hymexazol, and pyridaben) have been detected in the inland water and in fish (Kelly et al., 2018).

This ecosystem is home to a wide variety of freshwater fish, whose nutritional properties are of importance to local communities (Sroy et al., 2021). The Cambodian diet is based on rice and fish, the latter being consumed on a daily basis. The presence of contaminants in fish is an issue of nutrition and health. In particular, exposure to some potentially toxic elements during pregnancy is associated with abortion and abnormal fetal development (Ren and Shi, 2023; Pourret and Bollinger, 2017). The bioaccumulation of contaminants in fish tissue varies according to the aquatic environment, the properties of the contaminants, and their rate of absorption, deposition and excretion in the species. In general, the higher the concentration of a contaminant in the environment, the more fish accumulate it.

Several methods can be used to measure mineral elements in food. Inductively coupled plasma mass spectrometry (ICP MS) enables the simultaneous identification and quantification of major elements and ultra-traces in complex matrices based on their mass. Techniques combining Ultra-High Performance Liquid Chromatography and atomic spectroscopy have the advantage of rapidly and efficiently separating elements from complex samples, as well as assessing their chemical and speciation forms. Indeed, the toxicity of certain elements depends not only on their total content in the food, but also on their chemical forms, which will determine their mobility, toxicity and bioavailability (Rangasamy et al., 2020). For example, many arsenic species are present in freshwater fish including arsenobetaine, AsIII, AsV, monomethylarsonic acid, and dimethylarsinic acid as well as unknown arsenic species unidentified chemical structures (Hoy et al., 2023). Indeed, these complex sample matrix pose analytical challenges. Inorganic trivalent arsenic (As^{3+}) and pentavalent arsenic (As^{5+}) are the most toxic forms, while other common forms, including monomethyl arsenic and dimethyl arsenic would have lower toxicities (Rangasamy et al., 2020).

The performance of inland fisheries is intimately linked to the quality and quantity of water, as well as the size of the habitats in which fish carry out their life cycle (FAO, 2022). Pollution, climate change and intensive fishing techniques are threats not only to the wild fish stocks that live in inland ecosystem, but also to the aquaculture sector. Inland freshwater ecosystems are sensitive to environmental changes. Climate change and the building of hydroelectric dams are altering the course of the water. As a result, water levels in the ecosystem can fall, threatening the biological equilibrium of fish habitat. Concentrated pollutants can contribute to increase fish mortality as well as their contamination. In the interests of national food security and income generation, this sector aims to grow by a further 15 % a year through intensification and mass production, despite the fact that the TSL ecosystem is already fragile (FAO, 2020). The adoption of sustainable aquaculture practices is a challenge and technical innovation, institutional frameworks, human and economic resources have to be strengthened (Joffre et al., 2021).

This research aimed to compare the quality of three species of fish sampled in three different areas of the TSL and originating from the wild ecosystem or from cages in floating villages. The quality of the fish was determined by profiling nutrients (proteins, lipids, ash, minerals) and some contaminants (potentially toxic elements and mycotoxins). A food survey (24-hour dietary recall) estimated the fish and rice consumed by pregnant women. These data were used to estimate their risk of exposure to potentially toxic elements.

2. Materials and methods

2.1. Chemicals and reagents

Solvents and reagents were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France) pure standards of fatty acid methyl esters from Supelco (Sigma-Aldrich, Saint Quentin Fallavier, France) and polytetrafluoroethylene (PTFE) membranes from Sartorius (Palaiseau, France).

2.2. Sample collection

Freshwater fishes were collected from the wild TSL ecosystem and the cages of the floating villages located in Siem Reap, Battambang and Kampong Chhnang provinces (Fig. 1). The species selected are given here with their scientific, vernacular and

English names:

- Channa micropeltes (CMI): Trey Chhdor, Giant snakehead
- Pangasianodon hypophthalmus (PH): Trey Pra, Striped catfish
- Clarias macrocephalus (CMA): Trey Andieng, Catfish

Wild specimen were purchased from fishermen during fishing hours at each sampling site. Cage fishes were collected directly from the farmers by using a small net and a fishing rod. Approximately five kilograms of each fish species were collected which correspond to at least six fish specimens. The fish were grouped in pairs to obtain three representative samples for each location, species and production methods. The samples were placed in polyethylene bags and transported under ice to the laboratory and processed within 8–12 h.

2.3. Sample preparation and morphologic assessment

The morphometric characteristics were assessed on each fish. The weight and length were respectively determined with an electronic balance (Kern PCB 1000–1 Balance, Germany, precision ±0.1 g) and a tape meter. The fish were filleted and the edible parts (muscle, skin) were ground into small pieces using a meat grinder (HR-12, China) and stored at −20 °C for subsequent analysis. The analyses were carried out no more than six months after fish collection.

2.4. Determination of pH and titratable acidity

The pH value was determined by using a pH meter (Hanna, pH 213, Italy) (Tsighe et al., 2018). The titratable acidity was assessed by titration with sodium hydroxide (0.1 M) with phenolphthalein as indicator and expressed in g of lactic acid in 100 g of fresh samples (Horwitz, 2002).

2.5. Determination of proximate composition

The dry matter content was determined by drying 3 g of fresh samples in a thermostated oven (Memmert, UF B 500, Germany) at 105 °C for 24 h.

The ash content was determined by igniting 1.5 g dried samples in a muffle furnace (Nabertherm, P330, Germany) at 550 °C for 4 h (AOAC, 1990).

Total nitrogen content was assessed by the Kjheldal method. The crude protein content was calculated with a conversion factor of 6.25. The method has been validated using a reference sample and the uncertainty of the measurement is 2.5 % for nitrogen content.

The total lipid content was determined by previously published method with slight modifications (Folch et al., 1957). Briefly, 1.5 g of dried sample was hydrated with 10 mL of distilled water for 10 min and then dispersed in 30 mL of chloroform/methanol (2:1, v/v) for 2 min at 10 000 rpm (Ultra-Turrax T8, IKA, Germany). The mixture was sonicated for 5 min (Ultrasonic bath, Fisher Scientific, Germany), magnetically stirred (Heidolph, Reax 2, overhead shaker, Germany) for 1 h at room temperature and centrifuged at 2500 × g for 30 min at 4 °C (Avanti J-E, Beckman Coulter, France). The upper layer was discarded and the lower one was rinsed with 0.9 % NaCl and centrifuged again at 400 × g for 30 min at 4 °C. The organic layer was collected and evaporated at 40 °C (Genevac LTD, EZ-2 series, Sp Scientific, England). The total lipids (corresponding to the dry residue) were weighed. The maximum standard deviation for repeatability, determined with six repetitions on a same sample, was 4.0 %.

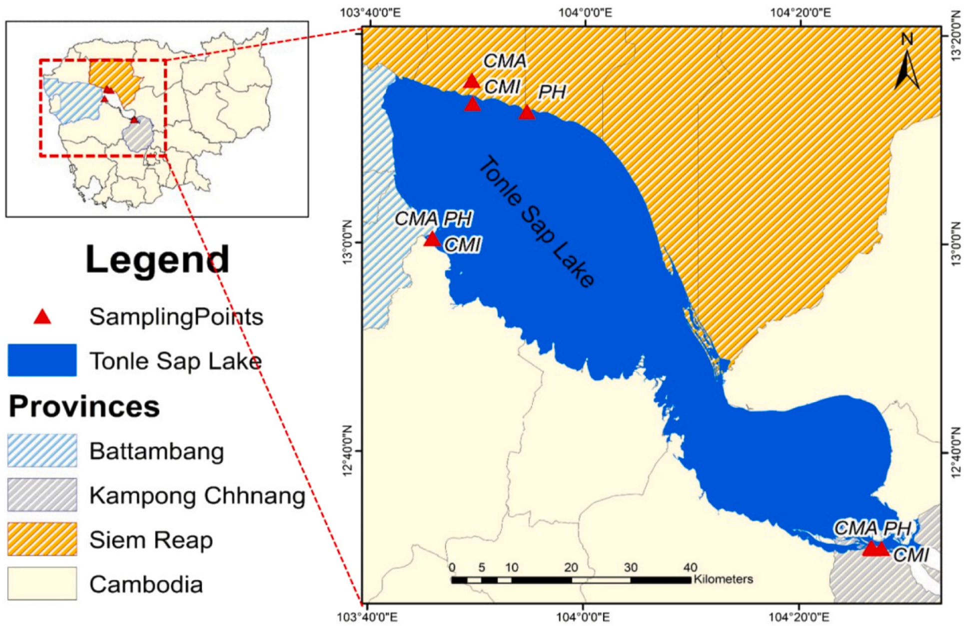

Fig. 1. Location of sampling sites in three provinces of Cambodia.

2.6. Fatty acid profiles

The fatty acids (FA) were esterified into methyl esters by acid catalyzed methylation (Servent et al., 2018). Briefly, 2 mL of sodium methoxide/methanol (0.8 % w/v) was added to 50 mg of the extracted lipid. The mixture was heated at 80 °C for 15 min, cooled down and neutralized with sulfuric acid 1 M (phenolphthalein as color indicator). Afterwards, the mixture was heated again at 80 °C for 5 min and 4 mL of saturated chloride solution was added accordingly. Fatty acid methyl esters (FAMEs) were extracted in 1 mL of hexane and analyzed by gas chromatography coupled with flame ionization detection (Agilent 6890 N, Agilent Technologies, Santa Clara, CA, USA) with a DB-WAX-Agilent column (100 m×0.25 mm×0.2 µm film, Sigma-Aldrich). FAMEs were identified according to their respective retention times compared with analytical standards.

2.7. Energy content

The energy content of fish was calculated based on their protein and lipid content which was expressed in kcal per 100 g of fresh sample using the conversion factors of 9 kcal and 4 kcal per 1 g of lipid and protein respectively (Rolls et al., 2005). Carbohydrates were considered to be in trace amounts.

2.8. Determination of mineral contents

The mineral contents were analyzed by inductively coupled plasma mass spectrometry (Thermo Elemental, X-Series, Germany) (Corns et al., 1993). Around 50 mg of the sample was digested with HNO_3:H_2O_2 (65:35, v/v) with a MARS Xpress microwave system (CEM Corporation, Mathews, NC, France). The digestion conditions were as follows: up to 120 °C for 15 min and then constant for 10 min; up to 160 °C in 20 min and constant for 15 min; finally, samples were cooled to 22 °C for 30 min and diluted to 25 mL with deionized ultrapure water. The methods were validated using certified reference materials ERM-BB422 (fish muscle), LGC7164 (crab paste) and NRC-SLRS-6 (river water) from LGC (Teddington, United Kingdom). The uncertainty of the measurement is about 10 %.

The limits of detections were in µg per g: 22.1 for calcium; 8.6 for potassium; 5.4 for sodium; 9.8 for phosphor; 3.4 for zinc; 2.6 for iron; 0.5 for magnesium; 0.2 for aluminum; 0.2 for copper; 0.02 for chromium; 0.02 for nickel; 0.05 for lead 0.01 for manganese; 0.003 for total arsenic; 0.001 for cadmium and cobalt.

The limit of quantification were in µg per g: 321.0 for calcium; 39.0 for potassium; 14.0 for magnesium; 35.0 for sodium; 97 for phosphor; 61.9 for iron; 30.7 for zinc; 7.7 for aluminum; 0.021 for cadmium; 0.36 for cobalt; 0.18 for chromium; 2.11 for copper; 0.4 for manganese; 1.85 for nickel; 8.82 for lead and 0.031 for total arsenic (tAs).

Total mercury (tHg) was quantified after the combustion of samples (approximately 50 mg) at 750 °C. Mercury vapors were retained on a gold trap and tHg concentrations were determined with a mercury analyzer (Leco, France). The limits of detections were 0.005 µg per g for tHg.

Methylmercury (MeHg) and inorganic arsenic (iAs) were estimated from the tHg and tAs contents. In fish, MeHg and iAs account for 92 % of tHg and 10 % of tAs, respectively (Kelly et al., 2018).

2.9. SAIN and LIM nutritional scores

The SAIN and LIM nutritional scores are used to compare foods according to their content of "positive" nutrients whose consumption should be encouraged and "negative" nutrients whose consumption should be limited (Darmon et al., 2009). The SAIN score represents the nutrient density obtained by the unweighted arithmetic mean of the percentage adequacy for 15 positive nutrients as follows:

$$\mathrm{SAIN} = \frac{\sum_{i=1}^{i=15} \mathrm{ratio_i}}{15} \times 100$$

$$\textit{With ratio}_\mathrm{i} = \left[\frac{\mathrm{nutrient_i}}{\mathrm{RV_i}}\right] \times \frac{100}{\mathrm{E_i}}$$

where nutrient i is the quantity (g, mg or µg) of positive nutrient i in 100 g of fish, RVi is the daily recommended value for nutrient i and Ei is the energy (in kcal) in 100 g of fish. The nutrients used to calculate SAIN were protein, fiber, vitamin C, vitamin E, thiamine (vitamin B_1), riboflavin (vitamin B_2), vitamin B_6, folate (vitamin B_9), calcium, iron, magnesium, zinc, potassium, α-linolenic acid (ALA), and docosahexaenoic acid (DHA). The contents of vitamins C, E, B_1, B_2, B_6 and B_9 were obtained from the literature (Rehbein and Oehlenschlager (2009).

The LIM score is the average contribution of 100 g of food to the maximum recommended values for three disqualifying nutrients (sugar, salt, saturated fat), whose intakes should be limited in a healthy diet. The LIM score was calculated for 100 g of fish as follows:

$$\mathrm{LIM} = \frac{\sum_{i=1}^{i=n} \mathrm{ratio}_i}{n}$$

$$\text{with } \mathrm{ratio}_i = \left[\frac{\mathrm{nutrient}_i}{\mathrm{MRV}_i}\right] \times 100$$

where $\mathrm{nutrient}_i$ is the quantity (g, mg or μg) of disqualifying nutrient i in 100 g of food, MRV_i is the daily maximal recommended value for nutrient i, and n is the amount of nutrient chosen for the calculation LIM 3. Added sugars were equal to zero as the fishes were not submitted to any formulation.

Food can be classified according to their (SAIN, LIM) scores into four groups (Darmon et al., 2009):

– Group 1: SAIN > 5 and LIM < 7.5 - food recommended for health
– Group 2: SAIN < 5 and LIM < 7.5 - neutral food
– Group 3: SAIN > 5 and LIM > 7.5 - food to consume in small quantities
– Group 4: SAIN < 5 and LIM > 7.5 - food to avoid or limit.

2.10. Assessment of the pregnant women diet

A survey was implemented by UNICEF in June 2017 to assess the food consumption of pregnant women in two districts of northeast Cambodia (Chetr Borei, Kratie). The ethical approval for this survey was obtained by UNICEF from the Cambodia National Ethical Committee for Health Research under the umbrella of "MyHealth" survey. The study was carried out in accordance to the Declaration of Helsinki for the protection of human subjects involved in research including confidentiality of personal information. To be eligible, the women had to eat normally and not participate to any celebration the previous day. Before the survey, the women were asked to participate freely to the interview at their household and signed a consent form.

A list of pregnant women was obtained from nine health centers administered by the Ministry of Health of Cambodia. More precisely, 643 eligible pregnant women between 1 and 35 weeks were identified in 67 villages. Sampling was done by randomization and initially targeted 180 pregnant women, but only 151 were selected. To protect personal data, an anonymous code was assigned to each woman. The 151 pregnant women took part in a 24-h dietary recall to index all type and quantities of food and liquids consumed the previous day. The survey was realized twice per women during two visits in one month during rainy season. The questionnaire was adapted to the Cambodian context using existing methodologies (Gibson and Ferguson, 1999). Women estimated the average quantities of food consumed using direct food weighing, estimation of the ingested quantity according to the weight bought at the market or the estimation of ingested quantity by calibrated household utensils (spoons, ladles, cups, bowls, plates). Ingredients ingested daily were calculated using R software.

2.11. Estimation of iron, zinc and potentially toxic elements daily intakes

Estimated daily intakes (EDI) of iron, zinc and potentially toxic elements from fish were calculated using the mineral contents obtained in our study (Section 2.8), as well as the fish intakes assessed for 151 pregnant women over one day (Section 2.10). EDI were expressed in μg per day.

To determine whether an element from food sources is considered likely to present a risk of adverse effects on human health, expert groups from Europe or FAO/WHO define the provisional tolerable monthly intake (PTMI), provisional tolerable weekly intake (PTWI) or benchmark dose lower confidence limit (BMDL) (JECFA, 1999; EFSA, 2009a; EFSA, 2009b; JECFA, 2011; EFSA, 2013). These values are expressed in μg per kg of body weight per month or week.

Average body weight of Southeast Asian women and average weight gain during pregnancy were assumed to be 50 kg and 12 kg respectively (Agusa et al., 2007). We have therefore calculated tolerable daily intakes for an average body weight of 62 kg for pregnant women and compared the EDI from fish to these reference values expressed per day (Table 4).

2.12. Statistical analysis

The proximate composition is expressed as mean ± standard deviation of triplicate experiments. Statistical analysis were performed using one-way analysis of variance (one-way ANOVA) using Excel. Significance was accepted at probability $P < 0.05$. The comparisons of means were performed using the Tukey test. The potentially toxic elements, iron and zinc daily intakes are presented per day in median [min – max].

3. Results and discussion

Once we had obtained all the data, we performed a one-way analysis of variance to see if location had a significant impact on them. As the P-values were always greater than 0.05, it was concluded that sampling location had no significant effect on the assessed parameters. Therefore, the data for each parameter for each location have been grouped together and are presented like this in the following sections. Furthermore, as no mycotoxins were detected in the fish regardless of the production method we have not integrated a specific table to report them (data not shown).

3.1. Fish morphometric, pH, titratable acidity and proximate composition

Channa micropeltes (*CMI*) is the largest species (Table 1). When a significant difference was observed, the size and weight of the fish were always greater for those produced on the farms. These results are in line with a previous study in Bangladesh, which found that the weight and size of fish were higher on farms than in the wild (Azim et al., 2002).

The pH and acidity results showed that the fish were fresh and that transport and cold storage were efficient enough to preserve fish from alteration (Mohanty et al., 2019; Sroy et al., 2021). For two species, the water and protein contents of the fish were similar regardless of production method. Protein levels range from 17.6 % to 20.1 %, and insofar as these levels are above 13 %, all three fish species are considered good sources of protein (Ullah et al., 2022). Fish are also rich in essential amino acids such as lysine, taurine and methionine (Kim, 2020).

The total lipid contents ranged from 2.0 % to 7.9 % according to fish species. The lipid contents of cage-fish were always significantly higher than that of wild fish for all species ($P<0.01$), and the increase varied from 38 % to 90 %. *PH* and *CMA* can be classified as medium fat fish as their content is within the range of 4–8 % (Rincón-Cervera et al., 2019). These results are in agreement with previously published data (Cahu et al., 2004; Oztekin et al., 2018). The high lipid content of farmed fish could be due to the higher fat content of their meals (industrial pellets), the frequency with which they are fed and their limited physical activity as a result of being caged (Klahan et al., 2023). The pellets used by farmers in Cambodia contain between 5.0 % and 6.5 % fat (data not shown).

As a consequence, the energy contents per 100 g were significantly higher for cage fish and this is probably due to the higher lipid contents of their daily diet.

The ash content varied between 0.9 % and 1.4 % in agreement with previous research (Sroy et al., 2021). The levels are somewhat lower than those already published by other authors probably because only fillets were analysed. Indeed, *PH* species is always eaten without the organs and bones; for *CMI* or *CMA* it depends on the household. Minerals

Table 1
Morphometry and physico-chemical characteristics of the three fish species.

	Channa micropeltes		*Pangasianodon hypophthalmus*		*Clarias macrocephalus*	
Parameters	Wild	Cage	Wild	Cage	Wild	Cage
Weight (g)	627.3±328.0*	843.3±272.0*	209.3±205.8***	701.6±246.4***	106.2±57.3	139.6±86.8
Length (cm)	39.0± 6.0	42.4± 4.3	27.3 ± 7.9***	43.4±6.2***	23.9 ±3.2	24.6±4.9
pH	6.7±0.1	6.7±0.1	6.6±0.2	6.7±0.1	6.9±0.1*	6.8±0.1*
Titratable acidity (%)	0.4±0.1	0.4±0.1	0.4±0.1*	0.3±0.1*	0.3±0.1*	0.4±0.5*
Moisture (%)	77.2±2.4*	74.8±3.1*	75.6±6.6	76.4±3.8	76.1±5.4	74.4±2.3
Proteins (g/100 g)	18.6±2.1*	20.1±1.2*	17.6±1.4	18.3±0.9	17.8±2.0	18.0±2.4
Lipids (g/100 g)	2.0±0.7***	3.8±0.7***	4.4±1.1***	6.0±1.3***	4.3±0.4***	7.9±1.1***
Energy (kcal/100 g)	92.7±10.0***	115.4±8.6***	109.2±9.7**	127.4±12.0**	110.6±9.4***	143.2±11.7***
Ash (g/100 g)	1.2±0.1***	1.4±0.17***	0.9±0.1***	1.2±0.2***	1.0±0.2*	0.9±0.1*

Results are expressed as mean ± standard deviation per 100 g of fresh weight. Different number of stars in the same row of each fish species mean a significant difference (**P*<0.05, ***P*<0.01, ****P*<0.001) by Tukey's test throughout the comparison of wild versus farmed fish.

are highly concentrated in fish bones, heads, gills and livers (Varol and Sünbül, 2019). Levels differed significantly according to production methods and species with no generalisable trend.

3.2. Fatty acid profiles

The FA profiles of the three species are presented in Table 2 and FA representing less than 1.5 % of total FA are not shown. Overall, 34 FAs were identified and constituted about 77.5–96.7 % of total FA. As already observed in other studies, SFA were predominant, followed by total monounsaturated fatty acids (MUFA) and total polyunsaturated fatty acids (PUFA) (Rincón-Cervera et al., 2019). The SFA content was significantly lower in cage-fish (for *PH* and *CMA* species) and the PUFA levels were significantly higher. Whatever the species, the main SFA and MUFA were palmitic acid (C16:0) and oleic acid (C18:1) in agreement with previous studies (Sroy et al., 2021).

All the fishes studied are rich in omega 3 fatty acids as the sum of EPA and DHA is above 80 mg/ 100 g or 100 kJ (Bucchini, 2019). They are also interesting for pregnant and lactating women for which it is recommended to ingest at least 300 mg of EPA and DHA per day (Simopoulos et al., 1999).

In Cambodia, 18.6 % of the population is below the poverty line, and people in rural areas do not have daily access to healthy diet every day (Vilain and Baran, 2016). The healthcare system is not accessible everywhere particularly in rural area and the promotion of healthy diet is essential (Liverani et al., 2017). Adequate consumption of EPA and DHA can protect them from undernutrition, improve their immune response and the development of fetus (Kris-Etherton et al., 2009).

Freshwater fish are unique sources of essential FA, EPA and DHA. However, despite the well-known benefice of fish consumption, pregnant and breastfeeding women are urged to limit their consumption, or even ban it, due to long-held beliefs and potential risks (Wallace et al., 2014). These practices are deleterious as EPA and DHA are an important factor in the retina and brain development of fetus in the last trimester of pregnancy and young children (Osendarp, 2011). Insufficient levels of DHA during child growth can induce congenital disorder (Martinez, 2001).

3.3. Mineral composition and potentially toxic elements

The mineral profiles were only determined for the two most popular fish species *CMI* and *PH* in Cambodia. The contents of several major elements (Ca, K, Mg and P) were higher in the cage-fish than in the wild-ones (Table 3). *PH* caged-fish are fed with pellets known to be rich rich in Ca (1.2–2.0 %) and P (1.2–1.5 %) (data not shown). *CM* raised in cages are fed on waste fish or specific manufactured feeds. These waste fish are small, inexpensive and locally harvested from the inland capture fishery (Joffre et al., 2021).

Table 2
Fatty acid profile expressed in percentage (%) of total fatty acids in the three fish species.

	Channa micropeltes		Pangasianodon hypophthalmus		Clarias macrocephalus	
Fatty acids (%)	**Wild**	**Cage**	**Wild**	**Cage**	**Wild**	**Cage**
C14:0 (myristic acid)	3.8±0.3	3.8±0.2	4.6±0.5	4.2±1.2	2.8±0.3	2.7±0.4
C15:0 (pentadecanoic acid)	4.8±2.2	5.4±1.3	5.3±2.0**	2.6±1.8**	2.4±0.2	2.0±0.6
C16:0 (palmitic acid)	23.2±1.7	21.9±0.8	25.9±1.4	24.5±2.8	24.2±1.2	24.5±0.8
C17:0 (heptadecanoic acid)	2.4±0.3	2.4±0.2	1.9±0.5	1.6±0.7	2.9±0.2***	1.3±0.2***
C18:0 (stearic acid)	7.5±0.5	7.3±0.6	7.2±0.7	7.3±1.0	8.6±0.7*	7.6±1.0*
C16:1 (palmitoleic acid)	9.1±1.6**	7.0±1.2**	7.9±1.0	6.1±2.7	5.4±0.7	5.8±0.8
C18:1n9 (oleic acid)	16.5±2.2	17.7±2.4	20.2±3.5	21.9±7.3	22.2±1.9	23.6±0.6
C18:2n6 (linolenic acid)	4.0±0.8	4.4±0.2	4.1±0.3**	7.1±2.9**	4.1±0.2***	5.0±0.3***
C22:2 (docosadienoic acid)	2.8±1.1	3.8±1.1	4.1±1.2	4.9±1.7	5.9±1.8***	9.3±1.5***
C18:3n3 (α-linolenic acid)	3.7±0.7	3.8±0.3	4.1±0.5**	3.0±0.7**	0.5±0.1***	2.7±0.3***
C20:5n3 (eicosapentaenoic acid)	0.7±0.1	0.7±0.1	1.6±0.5*	0.9±0.2*	1.7±0.3***	1.1±0.1***
C22:6n3 (docosahexaenoic acid)	5.3±1.2***	3.3±0.3***	2.6±0.8	3.1±0.1	2.6±1.1*	3.7±0.2*
SFA	41.6±2.6	40.7±0.8	44.9±2.4**	40.3±2.6**	40.9±1.4***	38.1±1.3***
MUFA	25.6±2.5	24.8±3.4	28.1±2.8	28.1±4.8	27.5±2.4	29.4±1.1
PUFA	16.6±0.9	16.1±0.8	16.5±1.5***	19.0±2.0***	14.8±1.6***	21.8±1.4***
PUFA/SFA	0.4±0.06	0.4±0.07	0.4±0.0*	0.5±0.1*	0.4±0.0***	0.6±0.0***
n-6 PUFA	5.5±0.7	5.8±0.3	4.7±0.5**	8.6±2.5**	5.2±0.5***	6.2±0.5***
n-3 PUFA	14.3±1.4***	10.6±0.8***	11.8±2.6	10.4±0.5	10.2±2.2*	7.6±1.8*
n-6/n-3	0.4±0.1***	0.5±0.1***	0.4±0.1***	0.8±0.3***	0.5±0.1**	0.9±0.2**
EPA+DHA	6.0±1.1***	4.1±0.3***	4.2±1.1	4.0±0.3	4.3±1.4	4.8±0.2

Results are expressed as mean ± standard deviation in percentage (%). Different number of stars in the same row of each fish species mean a significant difference (**P*<0.05; ***P*<0.01; ****P*<0.001) by Tukey's test throughout the comparison of wild versus farmed fish. SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids; EPA: eicosapentaenoic acid. DHA: docosahexaenoic acid. n-6 PUFA is the sum of C18:2n6, C20:3n6 and C20:4n6. n-3 PUFA is the sum of C18:3n3, C20:3n3, C20:5n3 and C22:6n3.

Table 3
Mineral profiles of the fillet of two fish species.

	Channa micropeltes		*Pangasianodon hypophthalmus*	
Major elements	Wild	Cage	Wild	Cage
K (mg/100 g)	373.1±27.7	386.2±23.5	221.9 ±15.5***	323.7 ±57.9***
P (mg/100 g)	193.3 ±18.1*	217.8 ±14.2*	133.8±6.3**	178.1±31.5**
Ca (mg/100 g)	52.3±5.2**	83.9 ±19.0**	40.7±5.4	30.2±18.0
Na (mg/100 g)	42.0±4.2	45.6±3.7	43.2±7.4	49.0±5.7
Mg (mg/100 g)	26.9±2.3	28.3±1.5	17.1±1.0**	21.5±2.6**
Trace elements and potentially toxic elements				
Zn (µg/100 g)	524.8±59.5	516.7±69.0	707.5±58.7	708.9±98.0
Fe (µg/100 g)	383.8±60.9	577.4 ±253.7	840.1±522.2	458.9±72.6
Al (µg/100 g)	11.6±20.7	32.5±33.3	509.5±694.0	14.4±13.1
Mn (µg/100 g)	43.4±11.9	49.4±9.8	34.5±12.5	26.7±8.2
Cr (µg/100 g)	13.4±6.0	33.2±38.3	20.2±17.2	8.4±3.1
Cu (µg/100 g)	10.6±1.4	10.3±2.0	20.1±7.8	19.8±3.0
Co (µg/100 g)	1.4±0.9	0.7±0.3	1.2±0.6	1.0±0.4
Ni (µg/100 g)	0.7±0.4	1.6±1.8	2.0±2.5	0.8±0.8
Cd (µg/100 g)	0.04±0.07	0.02±0.02	0.49±1.02	0.04±0.01
Pb (µg/100 g)	1.5±1.4	1.7±0.6	0.8±0.5	0.6±0.2
tHg (µg/100 g)	3.9±1.6	4.2±0.7	2.1±0.8*	1.1±0.5*
MeHg (µg/ 100 g)	3.6±1.5	3.9±0.7	1.9±0.7	1.0±0.5
tAs (µg/100 g)	1.2±0.5	1.0±0.2	1.6±0.3	1.2±0.7
iAs (µg/100 g)	0.1±0.1	0.1±0.0	0.2±0.0	0.1±0.1

Results are expressed as mean ± standard deviation per 100 g of fresh weight. tAs: total arsenic. iAs: inorganic arsenic. tHg: total mercury. MeHg: methylmercury. Different number of stars in the same row of each fish species mean a significant difference (*P<0.05; **P<0.01; ***P<0.001) by Tukey's test throughout the comparison of wild versus farmed fish.

The production method has no significant influence on the levels of minor elements and potentially toxic elements, with the exception of mercury in *PH* species. In the latter case, the mercury content of fish from the wild ecosystem was higher than that of specimens collected from fish farms ($P<$ 0.03). Caged fish are confined to a fixed and controlled area, usually close to houses in floating villages. In contrast, wild fish swim freely in the water of inland lakes and, as a result, have a greater risk of being located in an area contaminated by one or more sources of pollutants (Shaabani & Farsad, 2022). Indeed, mining, improper waste disposal, industrial waste and agricultural practices are known to be the main sources of potentially toxic elements (Yoshimura et al., 2022).

3.4. SAIN and LIM nutritional profiling

The SAIN indicator corresponds to a nutritional density calculated by the arithmetic mean of the percentage of adequacy for 15 positive nutrients, in order to obtain an overall assessment of nutritional quality (Darmon et al., 2007). Given their positioning in the group 1 (SAIN > 5 and LIM < 7.5), the fish species are recommended for health whatever their production system (wild ecosystem, caged system) (Fig. 2). These results confirm that the production method has a limited influence on the overall the nutritional profile of fish. One of the limitations of this nutritional profiling is that LIM indicator only reflects the nutritional risks linked to SFA, added sugars and sodium over the long term. It does not include potentially toxic elements in its calculation. Furthermore, its calculation is based on 100 g consumed, whereas local consumers may consume much higher quantities of certain food categories. For this reason, we wanted to take into account the dietary behaviour of Cambodian pregnant women as their fish intakes could be higher than 100 g per day.

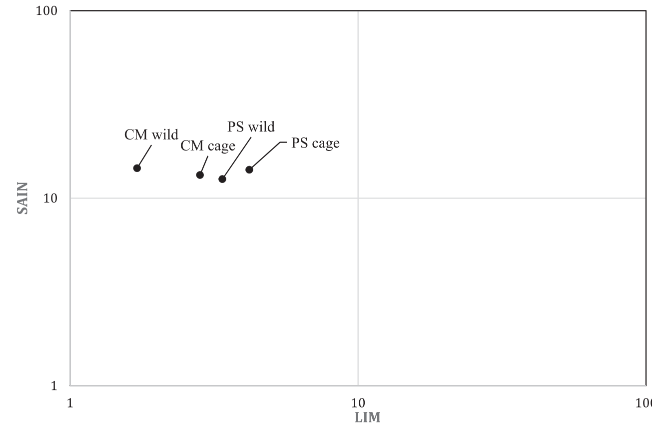

Fig. 2. Classification of the fish species sampled in cages or in the wild ecosystem according to SAIN–LIM scores. X axis represents LIM and Y axis represents SAIN.

3.5. Estimated daily intakes from fish

The fish consumption data for 151 pregnant women were determined at two distinct periods (Table S1, supplementary material). The mean consumption of fish per woman per day was 108 g, i.e. an annual consumption of 39.4 kg. These values are slightly higher than previous estimates, which reported annual consumption at 30.9 kg per capita at national level (FAO, 2019). It is important to note that some women consumed quantities of up to 718 g in a single day. Furthermore, the median rice intake per day reached 464 g (Table S1, supplementary material).

These results clearly show that it is extremely important to characterize the diversity of dietary behaviors and to have access to the quantities ingested, so as not to reason only theoretically on the basis of 100 g consumed per person per day. It is clear that some women exceed this intake by a considerable margin, both for fish and rice.

Iron and zinc median EDI from fish varied from 0 to 0.31 mg and 0–0.67 mg per day; these intakes contribute to micronutrient intakes of women in rural populations but these intakes would have been much higher if the fish studied had been consumed whole with their liver and head.

For all the assessed potentially toxic elements, median EDI from fish by these women were below the daily tolerable intakes defined by several international expert panels (Table 4). EDI intakes from fish are of the same order as those already calculated by other authors (Kelly et al., 2018), but higher for mercury and lead. In our study, only fish fillets were analyzed, as the species studied are mainly consumed by local communities in this way. Therefore, the results would probably have been slightly different for species consumed whole or cooked filet. According to the culinary practices, the heavy metal load remains unchanged or decreased. Numerous factors influence the metal concentration in cooked fish such as metal speciation, the cooking method, cooking time and temperature. However, whatever the treatment applied, the highest loss in mercury accounts for 33 % (Jinadasa et al., 2021).

The impact of the diversity of fish portions consumed on the median mercury and arsenic EDI was calculated for each pregnant women according to their fish and rice consumption estimated twice (Table 4, Fig. 3). In the case of mercury, three women who have eaten large quantities of fish have mercury intakes exceeding the tolerable limit (29 µg per day) (Fig. 3). In the case of mercury, only three women who consumed rather large quantities of fish reached and exceeded tolerable daily limits.

When rice consumption is taken into account, the mercury intake exceeded the tolerable limits for five women. The increase in mercury intake is not so great when rice is included, because when women eat a

Table 4
Median estimated daily intakes of potential toxic elements, iron and zinc from two fish species according to the quantities of fish consumed by 151 pregnant women.

	Estimated median daily Intake (µg/d)[a]				Tolerable daily intakes (µg/d)[b]
	Channa micropeltes		*Pangasianodon hypophthalmus*		
	wild	cage	wild	cage	
Iron	307.04 [0.0–2757.2]	461.92 [0.0–4148.0]	672.08 [0.0 – 6035.3]	367.12 [0.0 – 3296.7]	49 600[b]
Zinc	419.84 [0.0–3770.2]	413.36 [0.0–3712.0]	566.00 [0.0 – 5082.7]	567.12 [0.0 – 5092.7]	62 000[b]
Cadmium	0.03 [0.0–0.3]	0.02 [0.0–0.1]	0.39 [0.0 – 3.5]	0.03 [0.0 – 0.3]	22[f]
Lead	1.20 [0.0–10.8]	1.36 [0.0–12.2]	0.64 [0.0 – 5.7]	0.48 [0.0 – 4.3]	221[g]
Manganese	34.40 [0.0–308.9]	39.52 [0.0–354.9]	27.60 [0.0 – 247.8]	21.36 [0.0 – 191.8]	3000[h]
Mercury from fish	3.12 [0.0–28.0]	3.36 [0.0–30.2]	1.68 [0.0 – 15.1]	0.88 [0.0 – 7.9]	29[d] 35[e]
Mercury from rice	4.3 [0.0–16.6]				29[d] 35[e]
Mercury from rice and fish	8.1 [0.0 – 34.0]	8.3 [0.0 – 36.2]	6.4 [0.0 – 21.5]	6.0 [0.0 – 19.1]	29[d] 35[e]
Total arsenic from fish	0.96 [0.0–8.6]	0.80 [0.0–7.2]	1.28 [0.0 – 11.5]	0.96 [0.0 –8.6]	133[c]
Total arsenic from rice	82.4 [0.0–320.7]				133[c]
Total arsenic from fish and rice	94.1 [0.0–324.4]	84.9 [0.0–322.9]	84.4 [0.0–324.3]	84.2 [0.0–323.4]	133[c]
Inorganic arsenic from fish	0.10 [0.0 – 0.86]	0.08 [0.0 – 0.72]	0.13 [0.0 – 1.15]	0.10 [0.0 –0.86]	18.6[c]
Inorganic arsenic from rice	20.6 [0.0 – 80.2]				18.6[c]
Inorganic arsenic from fish and rice	20.74 [0.0 – 80.44]	20.72 [0.0 – 80.39]	20.79 [0.0 – 80.53]	23.34 [0.0 – 80.44]	18.6[c]

[a] Estimated daily intakes presented as the median intakes of 151 pregnant women surveyed at two distinct periods [min-max].
[b] calculated per day for a 62 kg body weight according to the limit published by International groups of Expert. PTWI: provisional tolerable weekly intake. PTMI: provisional tolerable monthly intake. $BMDL_{0.5}$: benchmark dose lower confidence limit. bw: body weight.
[c] PTWI: 0.3 µg/kg bw (EFSAb, 2009).
[d] PTWI: 3.3 µg/kg bw (JECFA, 2011)
[e] PTWI: 4 µg/kg bw (JECFA, 2011).
[f] PTWI: 2.5 µg/kg bw (EFSA, 2009a).
[g] PTWI: 25 µg/kg (JECFA, 1999).
[h] Adequate intake (EFSA, 2013).

lot of rice, they eat less fish. There is a compensation of one food by the other in the food bowl. As rice contains almost four times less mercury than fish, the increase in intake through its consumption is not so high.

The situation is different for arsenic. When total or inorganic arsenic intakes from fish are calculated, their median values never exceed the tolerable daily limit, whatever the quantities ingested or the type of fish. This means that none of the women exceeded the tolerable limit through fish consumption. However, when rice intakes are integrated in the risk assessment, the EDI exceed the tolerable daily limits without even including fish.

Many metals are essential for living organisms, but at higher doses they may be toxic. Arsenic is a naturally occurring element that originates in soils and diffuses into water. Worldwide contamination of drinking water by arsenic has been recognized as a major problem for human health. Potentially toxic elements are transferred from one species to another via the food chain, and are ingested in fine by humans through their diet (Schenone et al. 2014). Methylmercury and inorganic arsenic, the most toxic forms of mercury and arsenic, are a public health issue because of their respective neurotoxic and carcinogenic effects. Several recommendations were published to limit the consumption of these elements by the global population especially for pregnant women due to the risk of abnormal fetal development (Tchounwou et al., 2012). Human long-term exposure to arsenic can form skin lesions, cancers, neurological problems, peripheral vascular disease, hypertension and diabetes mellitus (Schenone et al., 2014).

3.6. Limitations

Our study only includes fish and rice consumption when estimating the risk of exposure to potentially toxic elements. It would be necessary to use a total diet analysis including all other food categories and the water drunk by pregnant women. This would provide an overall assessment of the risk of maternal and fetal exposure to potentially toxic elements. However, we estimate that this work is a contribution to the assessment of the risks of arsenic and mercury exposure for pregnant women in Cambodia integrating real food consumption data. Furthermore, the nutrient composition of fish varies according to life cycle stage and season. However, these aspects were outside the scope of the current study.

Furthermore, the potentially toxic elements were analysed using ICP MS, which provides total contents without differentiating between organic and inorganic forms of these elements. It would be interesting to complete these analyses by UHPLC coupled with spectroscopy to assess the chemical form and speciation of these elements to better assess the risk associated with their ingestion by humans.

4. Conclusion

The quality of three fish species produced in cages or taken from the wild ecosystem was similar in terms of nutritional profile and assessed contaminants. In a reassuring way, none of the samples contained detectable mycotoxins. The only two parameters that differ according to the production method were: i) the higher fat content of cage fish, probably due to their richer diet and lower physical activity and ii) the

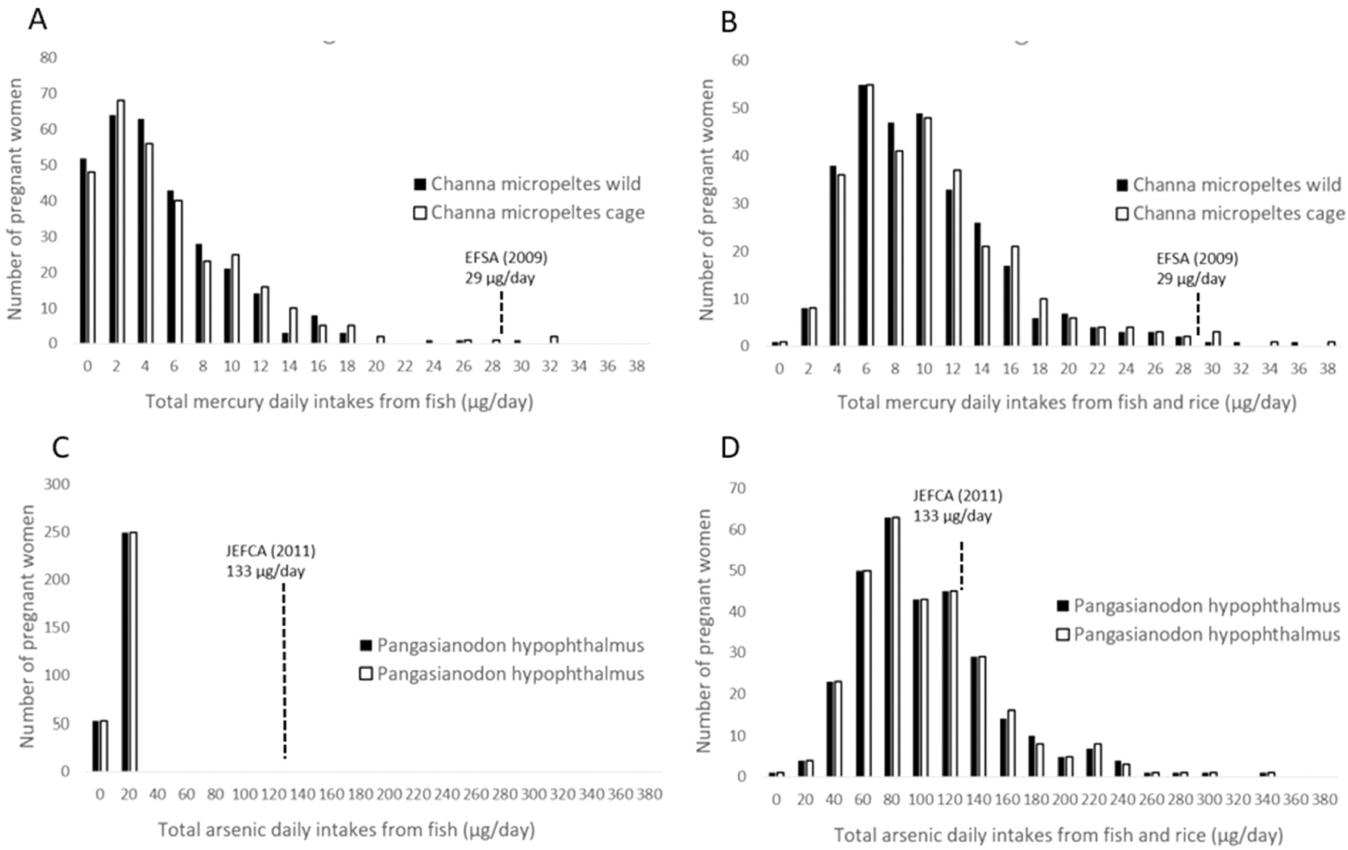

Fig. 3. Distribution of estimated daily mercury and inorganic arsenic intakes by 151 pregnant women through consumption of *Channa micropeltes* (A), *Channa micropeltes* and rice (B), *Pangasianodon hypophthalmus* (C), *Pangasianodon hypophthalmus* and rice (D). The fish and rice intakes were assessed during two surveys.

higher mercury content of *PH* fish from the wild ecosystem. Profiling by overall nutritional scores showed that all three fish species can be considered as nutrition and health-promoting food.

Using food consumption data from pregnant women, we found that median daily intakes of potentially toxic elements from fish were well below the tolerable limits published in the international literature by various groups of experts whatever the fish species and production method. Only mercury intakes from fish came close to the published tolerable daily limits and three women exceeded them.

Aquaculture is a promising strategy for improving access to fish for Cambodia's vulnerable populations. Fish quality is good, whatever the production method. However, it will be important for the public authorities to take steps to protect the Tonle Sap Lake ecosystem by developing public policies to limit its pollution and ensure that, in the decades to come, fish stocks meet demand and are still of good overall quality.

CRediT authorship contribution statement

Channmuny Thanh: Writing – review & editing, Writing – original draft, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sylvie Avallone:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Chanthol Peng:** Writing – review & editing, Validation, Resources, Investigation, Funding acquisition, Conceptualization. **Hasika Mith:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Charlie Poss:** Writing – review & editing, Formal analysis. **Adrien Servent:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Sophanith Phal:** Writing – review & editing, Validation, Investigation, Data curation, Conceptualization. **Arnaud Laillou:** Writing – review & editing, Visualization, Validation, Resources, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

None

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2024.106357.

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