Micronutrient Composition of 35 Food Fishes from India and Their Significance in Human Nutrition

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Abstract The micronutrients (vitamins and minerals) are required in small amounts but are essential for health, development, and growth. Micronutrient deficiencies, which affect over two billion people around the globe, are the leading cause of many ailments including mental retardation, preventable blindness, and death during childbirth. Fish is an important dietary source of micronutrients and plays important role in human nutrition. In the present investigation, micronutrient composition of 35 food fishes (includes both finfishes and shellfishes) was investigated from varying aquatic habitats. Macrominerals (Na, K, Ca, Mg) and trace elements (Fe, Cu, Zn, Mn, Se) were determined by either atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS)/atomic emission spectrometry (ICP-AES). Phosphorus content was determined either spectrophotometrically or by ICP-AES. Fat-soluble vitamins (A, D, E, K) were analyzed by high-performance liquid chromatography

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(HPLC). The analysis showed that, in general, the marine fishes were rich in sodium and potassium; small indigenous fishes (SIFs) in calcium, iron, and manganese; coldwater fishes in selenium; and the brackishwater fishes in phosphorous. The marine fishes *Sardinella longiceps* and *Epinephelus* spp. and the SIFs were rich in all fat-soluble vitamins. All these recommendations were made according to the potential contribution (daily value %) of the species to the recommended daily allowance (RDA). Information on the micronutrients generated would enhance the utility of fish in both community and clinical nutrition.

Keywords Micronutrients · Minerals · Vitamins · Food fishes

Introduction

Micronutrients are needed only in minuscule amounts but are essential for proper growth and development. And the consequences of their absence are severe. Therefore, the current practice of evaluating nutritive value of diets should include not only energy and protein adequacy but also the micronutrient (mineral and vitamin) density of the diet. Micronutrient deficiency is a form of malnutrition and is a recognized health problem in many developing countries; however, people from developed countries also suffer from various forms of micronutrient deficiencies and diets which lack adequate amount of minerals and vitamins lead to such diseases. Vitamin A, iodine, and iron are most important in global public health terms; their lack represents a major threat to the health and development of populations the world over, particularly children and pregnant women in low-income countries [1]. Approximately one third of the developing world's children under the age of



5 are vitamin A-deficient and therefore ill-equipped for survival, as vitamin A deficiency is often associated with protein calorie malnutrition (PCM). Iron deficiency anemia during pregnancy is associated with 115,000 deaths each year, accounting for one fifth of total maternal deaths [2]. Keeping this in view, micronutrient supplementation programs were incorporated as an integral part of Millennium Development Goals and their micronutrient initiative is providing mineral and vitamin supplements to the most vulnerable, i.e., women and children across the world [3].

India is home to more than 10 % of the global fish diversity. Presently, the country ranks second in the world in total fish production with an annual fish production of about 9.06 million metric tons [4]. Fish is an important dietary component and is a rich source of quality animal proteins [5], polyunsaturated fatty acids (PUFAs) [6], and micronutrients [7, 8]. Vitamin A from fish is more readily available to the body than from plant sources [9]. Fish especially oily ones have been found to be excellent sources of vitamin D [10]. Fish, in this context, can play a vital role as it is a rich source of micronutrients. Therefore, there is a need to generate and document nutritional information on the numerous varieties and species of food fishes available as our previous work reporting the amino acid composition of 27 food fishes from India [11]. The primary objective of the study was to generate information on micronutrient composition of important food fishes and shellfishes including macrominerals (Na, K, Ca, Mg, P), trace elements (Fe, Cu, Zn, Mn, Se), and fat-soluble vitamins (vitamins A, D, E, K). The secondary objective of the study was to enhance the scope for their utility in human nutrition by evaluating their potential contribution (daily value %) to the recommended daily allowance (RDA).

Materials and Methods

Collection of Sample

Fresh fishes and shellfishes were collected from the landing stations and were brought to the laboratory in ice. The study did not include any live animal. For mineral analysis, all total 35 species of fishes and shellfishes (crustaceans and mollusc) from different habitats were studied (Table 1). The length (in cm) and weight (in g) of individual fish were measured and recorded. Scales were removed by scraping, with the edge of a knife with a titanium blade; the blade was rinsed with distilled water, and fillets were removed and freed of the bones. The size of these fishes ranged between 500 and 800 g per fish except small indigenous fishes (SIFs) and shellfishes. SIFs are those which grow to a maximum length of about 20 cm on maturity or adult stage [12, 13].

For larger fishes, 16 individual fish samples were taken for sample preparation and analysis. In case of the SIFs and shellfishes, pooled samples were prepared (each pooled sample contained 100 individuals); six such pooled samples were prepared for each species. The samples were stored at -40 °C preceding analysis, wherever necessary, and all the samples were analyzed in triplicate. For the SIFs/shellfishes, samples were pooled as the amount required for micronutrient analysis was not sufficient from individual fishes, and therefore, pooling 100 individual fishes was the better option. Further, the no. of pooled samples was restricted to six because of the constraint in getting the samples in higher amount.

Mineral Analysis by AAS

Mineral contents of the fish muscle were assayed using atomic absorption spectrometry (AAS). About 3–5 g of fish muscle tissue was taken in a conical flask, and a digestion mixture (3 ml HClO₄ + 21 ml HNO₃ + 1.5 ml H₂SO₄) was added and incubated overnight at room temperature. Further, it was heated for 2–3 h or until colorless. It was then filtered with Whatman paper no. 42 making the volume up to 100 ml with 2 % HNO₃ [14]. Minerals (Na, K, Ca, Mg, Fe, Cu, Zn, Mn, and Se) were estimated using Atomic Absorption Spectrophotometer (Varian Spectra-220 AA, Australia). Phosphorous was measured spectrophotometrically (Thermo Spectronic, UV1) by measuring the absorbance at 660 nm after color development with the formation of phosphomolybdic acid [15]. The mineral contents were expressed in mg/100 g of wet weight (minced edible part).

Mineral Analysis by ICP-MS/ICP-AES

For mineral analysis, the minced fishes were kept overnight in a hot air oven at 120 °C, and then, the dried samples were powdered in a mixer grinder and stored in aluminum foils. A microwave-assisted digestion procedure was carried out in order to achieve a shorter digestion time. Homogenized powders (0.5 g) were weighed in glass digestion bombs, and 3 ml suprapure HNO₃ (E. Merck) was added to the samples. The bombs were firmly closed and put in the microwave oven for digestion under controlled pressure. The basic program of the microwave digestion is given in Table 2. After digestion, the glass bombs were cooled and the mineralized samples were diluted to 50 ml with Milli-Q water and stored in a refrigerator. Mineral analysis was carried out by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Fisher X Series 2) or inductively coupled plasma atomic emission spectrometry (ICP-AES) (ICP spectrometer, iCAP 6300 Radial, Thermo Scientific). The microelements viz. iron, copper, zinc, manganese, and selenium were directly analyzed; however, the
 Table 1
 Analytical methods used for micronutrient composition of selected food fishes and shellfishes

Sl no.	Fish and shellfish species	Method reference		Habitat (source)	
		Mineral	Vitamin		
1.	Epinephelus spp.	AAS [14] ^a	HPLC [19]	Marine (capture)	
2.	Euthynnus affinis	do	nd	Marine (capture)	
3.	Katsuwonus pelamis	do	HPLC [19]	Marine (capture)	
4.	Leiognathus splendens	do	HPLC [19]	Marine (capture)	
5.	Nemipterus japonicus	do	nd	Marine (capture)	
6.	Rastrelliger kanagurta	do	nd	Marine (capture)	
7.	Sardinella longiceps	do	HPLC [19]	Marine (capture)	
8.	Stolephorus commersonii	do	nd	Marine (capture)	
9.	Stolephorus waitei	do	nd	Marine (capture)	
10.	Thunnus albacares	do	nd	Marine (capture)	
11.	Trichiurus lepturus	do	HPLC [19]	Marine (capture)	
12.	Ailia coila (SIF)	ICP/AES [17]	nd	Freshwater (capture)	
13.	Amblypharyngodon mola (SIF)	ICP/MS [16]	HPLC [19]	Freshwater (capture)	
14.	Anabas testudineus (SIF)	AAS [14] ^a	do	Freshwater (capture)	
15.	Catla catla	do	do	Freshwater (aquacultured	
16.	Cirrhinus mrigala	do	do	Freshwater (aquacultured	
17.	Clarias batrachus	do	do	Freshwater (capture)	
18.	Gudusia chapra (SIF)	ICP/AES [17]	nd	Freshwater (capture)	
19.	Heteropneustes fossilis	AAS [14] ^a	HPLC [19]	Freshwater (capture)	
20.	Labeo rohita	do	do	Freshwater (aquacultured	
21.	Puntius sophore (SIF)	ICP/MS [16]	do	Freshwater (capture)	
22.	Sperata seenghala	do	do	Freshwater (capture)	
23.	Tenualosa ilisha	do	do	Freshwater (capture)	
24.	Xenentodon cancila	ICP/AES [17]	nd	Freshwater (capture)	
25.	Lates calcarifer	AAS [14] ^a	nd	Brackishwater (capture)	
26.	Mugil cephalus	do	nd	Brackishwater (capture)	
27.	Cyprinus carpio	do	nd	Coldwater (capture)	
28.	Neolissochilus hexagonolepis	do	nd	Coldwater (capture)	
29.	Oncorhynchus mykiss	do	nd	Coldwater (capture)	
30.	Schizothorax richardsonii	do	nd	Coldwater (capture)	
31.	Tor putitora	do	nd	Coldwater (capture)	
32.	Penaeus monodon (prawn)	do	nd	Brackishwater (capture)	
33.	Fenneropenaeus indicus (prawn)	do	nd	Brackishwater (capture)	
34.	Crassostrea madrasensis (mollusc)	do	HPLC [19]	Marine (capture)	
35.	Perna viridis (mussel)	do	do	Marine (capture)	

do same as above, nd not determined, SIF small indigenous fish

^a Phosphorus content was analyzed spectrophotometrically [15]

macroelements (sodium, potassium, calcium, magnesium) were analyzed after appropriately diluting the mineralized samples. The ICP-MS and ICP-AES operating conditions are shown in Table 3 [16, 17]. A commercially available multielement stock standard solution was used, after appropriate dilution for instrumental calibration and sample spiking, respectively. Quantification was done in ICP-AES by comparing with multielement standard IV, MERCK (NIST) for sodium, potassium, calcium, magnesium, iron, copper, zinc, and magnesium and Trace CERT (NIST) for phosphorous. For ICP-MS, 1.09492.0100 and 1.09494.0100,

Table 2 Microwave digestion program			
Step	Power (W)	Ramp time (min)	Hold time (min)
1	300	05.00	05.00
2	400	10.00	10.00
3	600	10.00	05.00

E. Merck multielement stock standard solution was used, respectively.

The instrument limit of detection (LoD) for AAS, ICP-MS, and ICP-AES was calculated as the concentration associated with 3.3 times the standard deviation of the background noise recorded on nine measurements of the procedural blank and given in online resource 1 (Table S1).

Fat-Soluble Vitamin Analysis

Fat-soluble vitamins were analyzed by high-performance liquid chromatography (HPLC). Firstly, fish oil was extracted from fresh meat by homogenizing 30 g of fresh meat in 450 ml of chilled chloroform/methanol (2:1) mixture following the method described by Folch et al. [18]. About 150 mg fish oil was refluxed with 25 ml methanol and 150 % potassium hydroxide (KOH) in water bath for 30 min. Fat-soluble vitamins were extracted with 50 ml petroleum ether. The petroleum ether layer was collected, concentrated, and dissolved in 5 ml acetonitrile. Twenty microliters of sample was injected in HPLC (Shimadzu LC 10AS) equipped with C_{18} RP column and UV detector [19]. The mobile phase of HPLC consisted of acetonitrile (solvent A) and methanol (solvent B). A simple linear gradient system was used, starting from (solvent A/solvent B) 50/50 to 70/30 in 20 min. The mobile phase flow rate was 1 ml/min. The fatsoluble vitamins were identified and quantified by comparison with the retention times and peak areas of standards (Sigma-Aldrich). The vitamin contents were expressed in IU/100 g of wet weight (minced edible part).

Table 3 The ICP-MS operating
conditions and measurement
conditions

Data are presented in the form of mean \pm standard deviation (SD). One-way analysis of variance (ANOVA) at 0.05 level of significance was employed to compare the variation in micronutrients with respect to different species using MS Excel 2007. Further, one-way ANOVA followed by post hoc Tukey's HSD test was carried out by SPSS 16.0 at 0.05 level of significance.

Calculation of DV % and Potential Contribution to RDA

The potential contribution of fish and shellfishes to daily value of foods (DV %) was calculated from recommended daily allowance (RDA) for an adult man weighing 60 kg [20]. For example, the RDA of calcium is measured as intake/kg body weight, as 10 mg/kg of individual body weight. It means that an individual weighing 60 kg would need to take 600 mg (10×60) of calcium daily. Considering this RDA, consumption of 50 g fish containing 420.85 mg calcium (*Amblypharyngodon mola*) by an individual weighing 60 kg would fulfill 70.14 % of the daily calcium requirement. This is the DV % of 50 g of *Amblypharyngodon mola* with respect to calcium. Similarly, the DV % for the minerals considering the requirement and content of a particular nutrient in the selected fishes was calculated.

Results and Discussion

Micronutrients play a central role in metabolism and in the maintenance of tissue function, and there is a highly integrated system to control the flux of micronutrients in illness. An adequate intake therefore is necessary to sustain metabolism and tissue function and prevent mineral and vitamin deficiency. Micronutrient deficiency conditions relate to many chronic diseases, such as osteoporosis, osteomalacia, thyroid deficiency, colorectal cancer, and cardiovascular diseases [21]. Malnutrition and malabsorption are the main causes of the

Operating conditions	ICP-MS	ICP-AES
Spray chamber	Cyclonic quartz	Cyclonic glass
RF power (W)	1398	1300
Plasma gas flow (l/min)	13.0	15
Nebulizer gas flow rate (l/min)	0.98	0.80
Auxiliary gas flow rate (l/min)	0.5	0.5
No. of replicates	3	3
Isotopes	²³ Na, ³⁹ K, ⁴³ Ca, ²⁴ Mg, ⁵⁴ Fe, ⁵⁵ Mn, ⁶⁶ Zn, ⁶⁵ Cu	²³ Na, ³⁹ K, ⁴³ Ca, ²⁴ Mg, ⁵⁴ Fe, ⁵⁵ Mn, ⁶⁶ Zn, ⁶⁵ Cu

dearth in mineral and vitamins in the body. The primary deficiency occurs when there is paucity of these in the diet. This may be due to restrictive diets, food habits, poverty, and paucity of food sources of vitamins. Therefore, information on the micronutrient composition of foods serves as a basis for establishing their potential nutritive value and its utilization in specific deficiency diseases. Here, we report the micronutrient composition of 35 food fishes and shellfishes from the Indian subcontinent (Tables 4 and 5) which could be useful in patient counseling and recommending species for patients with specific requirements and thus could be useful in human nutrition. Fish species-specific richness in minerals and vitamins is listed in Table 6, and the potential contribution (DV %) to recommended daily allowance (RDA) for adult men of those fishes is given in Fig. 1.

Macrominerals

Macrominerals are inorganic elements (sodium, potassium, calcium, magnesium, phosphorous) that human body needs in large quantities to perform several important physiological functions; however, amounts needed in the body are not an indication of their importance. The sodium and potassium contents of these fishes were significantly different from each other (P < 0.05). Among the fishes and shellfishes studied, sodium contents were found to be highest in the green mussel Perna viridis (1810.2 mg/100 g) followed by Penaeus monodon (831 mg/100 g). The highest potassium was reported in the marine fish Rastrelliger kanagurta (2397 mg/100 g), but Nemipterus japonicus and Stolephorus commersonii were also very rich in sodium and potassium (Table 4). The sodium and potassium contents of these fishes were notably higher than those of the freshwater fish Tribolodon hakonensis [22] and Baltic herring [23] and the potassium content from that reported in sea bass (459.7 mg/100 g), sea bream (393.8 mg/100 g) [8], blue whittling (388 mg/100 g), and trout (306 mg/100 g) [24].

Calcium is one of the most abundant minerals of the body. It is needed for normal functioning of muscles and nervous system. It also plays an important role in blood clotting process. Calcium and phosphorus are essential elements for formation of the bones and teeth (formation and mineralization). Deficiency of calcium may be associated with rickets in young children and osteomalacia (softening of the bones) in adults and older people. Calcium and phosphorous content varied considerably with a range from 64.1 to 5310 and 113.2 to 3900 mg/100 g, respectively, much higher than that reported elsewhere for fish and shellfish [25]. The freshwater garfish *Xenentodon cancila* was found to be a rich source of both calcium and phosphorous, i.e., 5310 and 3900 mg/100 g, followed by freshwater small indigenous fish (SIF), *Gudusia chapra* and *Ailia coila*, respectively at P < 0.05. Moreover, the

other SIFs, *Puntius sophore* (944.6 mg/100 g) [26] and *Amblypharyngodon mola* (841.7 mg/100 g), were also very rich in calcium. As would be expected, calcium and phosphorus contents were much higher in species in which bones are commonly consumed and included in the edible part [25, 27]. The calcium content of *Amblypharyngodon mola* is comparable with earlier reported values by Ross et al. (853 mg/100 g) [12] and also comparable with some other SIFs like *Parambassis ranga* (955 mg/100 g) and *Esomus danricus* (891 mg/100 g) [12].

Magnesium is an essential component of bone and cartilage and is a co-factor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes. Magnesium content ranged between 27 to 281 mg/100 g, and the prawns *Penaeus monodon* (281.7 mg/100 g) and *Fenneropenaeus indicus* (252.0 mg/ 100 g) were found to be rich sources of magnesium, significantly higher than other fish species studied (P < 0.05), which is largely consistent with results reported earlier [28].

Microminerals

The microminerals (iron, copper, zinc, manganese, selenium, etc.) are required in trace amounts but are important for normal functioning of the body. Iron deficiency is the most common and widespread nutritional disorder in the world affecting 2 billion people of the world's population. The major health consequences include poor pregnancy outcomes, impaired physical and cognitive development, and increased risk of morbidity in children and reduced work productivity in adults [29]. Among the fishes and shellfishes studied, iron content was found to be significantly higher (P < 0.05) in the freshwater SIF Gudusia chapra (36.5 mg/100 g) followed by shellfish Penaeus monodon (16.41 mg/100 g) and brackishwater fish Mugil cephalus (12.8 mg/100 g). Other SIFs like Amblypharyngodon mola, Puntius sophore, and Ailia coila were also rich in iron (Table 4).

Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. Zinc stabilizes the molecular structure of cellular components and membranes and contributes, in this way, to the maintenance of cell and organ integrity. Furthermore, zinc has an essential role in polynucleotide transcription and, thus, in the process of genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all life forms [28]. Zinc content varied considerably from 0.4 to 26.0 mg/100 g. In our study, the marine fishes were found to be rich in zinc content, significantly higher (P < 0.05) in *Stolephorus waitei* (26.0 mg/

1able 4 Milleral composition of important lood fishes from india <u> <u> </u> </u>						Missioninouol				
r isii species	Macrolinneral					MICTORING				
	Na	K	Ca	Mg	P	Fe	Cu	Zn	Mn	Se
Marine										
Epinephelus spp.	195.8 ± 99.2^{1}	443.7 ± 45.9^{1}	162.1 ± 10.6^{1}	pu	1973 ± 99.8^{1}	$2.6 \pm 0.9^{1.2}$	pu	$1 \pm 0.5^{1,2}$	0.2 ± 0.0^1	0.12 ± 0.0^{1}
Euthynnus affinis	211 ± 0.0^2	2123 ± 0.0^2	nd	pu	nd	7.12 ± 0.0^3	nd	16.2 ± 0.2^3	nd	nd
Katsuwonus pelamis	63.4 ± 20.8^3	193.9 ± 54.8^3	206.3 ± 47.8^{2}	nd	698 ± 87.2^{2}	$2.7 \pm 1.4^{2,4}$	nd	$2.8\pm1.7^{4.5}$	0.1 ± 0.0^2	0.1 ± 0.0^1
Leiognathus splendens	286.6 ± 38.1^4	435.8 ± 48.3^4	330.8 ± 40.3^3	nd	1249 ± 70.8^3	$2.2\pm1.6^{1.5,6}$	nd	$2.5\pm1.1^{4.6}$	0.3 ± 0.0^3	0.2 ± 0.0^2
Nemipterus japonicus	364 ± 0.2^{5}	2334 ± 0.2^{5}	nd	nd	nd	4.0 ± 0.1^7	1.0 ± 0.0^1	$12.0\pm 0.0^{7,8}$	nd	nd
Rastrelliger kanagurta	107 ± 0.1^6	2397 ± 0.2^{6}	nd	nd	nd	5.0 ± 0.1^8	1.0 ± 0.0^1	13.0 ± 0.1^9	nd	nd
Sardinella longiceps	182.6 ± 10.7^7	268.9 ± 20.2^7	523.9 ± 45.6^4	nd	1389 ± 91.5^4	7.8 ± 1.2^9	nd	4 ± 2.3^{10}	0.4 ± 0.2^4	0.1 ± 0.1^1
Stolephorus commersonii [46]	362 ± 0.1^8	2347 ± 0.0^8	nd	nd	nd	4.0 ± 0.1^7	1.0 ± 0.0^1	21.0 ± 0.1^{11}	pu	nd
Stolephorus waitei	244 ± 0.0^9	2218 ± 0.0^9	nd	nd	nd	6.0 ± 0.0^{10}	1.0 ± 0.1^1	26.0 ± 0.1^{12}	pu	nd
Thunnus albacares	127 ± 0.01^{10}	2170 ± 0.0^{10}	nd	nd	nd	7.05 ± 0.0^3	2 ± 0.0^2	$12.1 \pm 0.2^{7.8}$	nd	nd
Trichiurus lepturus	205.6 ± 23.9^{11}	225.5 ± 21.5^{11}	159.7 ± 60.1^{5}	nd	1157 ± 89.2^{5}	$1.9\pm 0.9^{5,11}$	nd	1.3 ± 0.9^2	0.1 ± 0.0^2	0.3 ± 0.0^3
Freshwater										
Ailia coila	270.0 ± 50.2^{12}	120.0 ± 21.2^{12}	2410.0 ± 14.7^{6}	160.0 ± 12.3^{1}	1880 ± 45.2^{6}	10.9 ± 1.3^{12}	nd	10.2 ± 2.1^{13}	1.3 ± 0.9^{5}	nd
Amblypharyngodon mola	52.7 ± 6.5^{13}	211.3 ± 20.1^{13}	841.7 ± 40.2^{7}	40.2 ± 2.9^2	pu	11.9 ± 3.4^{13}	0.2 ± 0.0^3	3.9 ± 1.3^{10}	1.1 ± 0.4^6	nd
Anabas testudineus	236.8 ± 69.4^{14}	$178.3\pm\!8.8^{14}$	252.6 ± 45.7^8	pu	159.8 ± 3.5^7	$2.3\pm 0.8^{1,2,6}$	pu	$0.9\pm 1.0^{1,14}$	0.8 ± 0.4^7	0.3 ± 0.2^3
Catla catla	198.3 ± 31.9^{15}	283.9 ± 6.9^{15}	161.1 ± 4.4^9	pu	146.8 ± 2.1^{8}	$1.6\pm 0.4^{11,14}$	pu	$1.3\pm0.3^{13,15}$	0.3 ± 0.0^3	0.2 ± 0.1^2
Cirrhinus mrigala	205.7 ± 31.6^{16}	$273.4\pm\!24.0^{16}$	222.5 ± 15.7^{10}	pu	131.9 ± 3.3^9	$1.9\pm0.5^{5,6,11}$	pu	$1.1 \pm 0.6^{1,2}$	0.5 ± 0.3^8	$0.3 \pm 0.0^{4.5}$
Clarias batrachus	201.5 ± 69.2^{17}	$262.1\pm\!46.0^{17}$	222.3 ± 3.3^2	pu	129.4 ± 2.1^{10}	$2.2\pm0.3^{1,5,6}$	pu	$0.7\pm 0.1^{13,16}$	0.2 ± 0.1^1	0.4 ± 0.2^6
Gudusia chapra	220 ± 15.2^{18}	1140 ± 7.9^{18}	3440 ± 10.4^{11}	170 ± 9.8^3	2490 ± 32.1^{11}	36.5 ± 4.91^{15}	pu	12.3 ± 2.3^8	4.61 ± 1.3^{9}	nd
Heteropneustes fossilis	215.8 ± 17.8^{19}	$186.4\pm\!48.4^{19}$	164.4 ± 21.5^{12}	nd	113.2 ± 8.3^{12}	$2.4 \pm 0.5^{1.2}$	nd	1.2 ± 0.3^{2}	0.2 ± 0.0^1	0.1 ± 0.0^1
Labeo rohita	202.1 ± 44.5^{20}	267.5 ± 32.9^7	205.7 ± 4.8^{13}	nd	124.9 ± 10.5^{13}	$2.2\pm 0.5^{1.5,6}$	nd	1.9 ± 0.6^{17}	0.4 ± 0.2^4	0.6 ± 0.0^7
Puntius sophore	57.8 ± 4.5^{21}	197.9 ± 28.9^{20}	944.6 ± 55.4^{14}	37.8 ± 1.1^4	nd	11.6 ± 3.6^{12}	0.1 ± 0.0^3	$5.4\pm 0.4^{18,19}$	1.1 ± 0.7^6	nd
Sperata seenghala	43.4 ± 7.1^{22}	278.5 ± 47^{21}	64.1 ± 21.6^{15}	27 ± 3.9^{5}	nd	$1.3\pm 0.5^{14,16}$	0.3 ± 0.2^4	0.4 ± 0.1^{20}	0.1 ± 0.0^2	nd
Tenualosa ilisha	63.1 ± 7.7^{3}	493.7 ± 17.4^{22}	119 ± 45.7^{16}	38.3 ± 13.3^6	nd	3 ± 0.7^{4}	0.3 ± 0.0^4	$0.9\pm 0.0^{1,14}$	0.3 ± 0.0^3	nd
Xenentodon cancila	250 ± 24.2^{23}	1080 ± 47.8^{23}	5310 ± 23.5^{17}	220 ± 15.3^7	3900 ± 49.6^{14}	7.51 ± 1.9^3	1.32 ± 0.3^{5}	21.3 ± 3.6^{11}	1.47 ± 0.9^{10}	nd
Coldwater										
Cyprinus carpio	268 ± 15.2^{24}	1125.6 ± 36.8^{24}	409.3 ± 25.6^{18}	nd	nd	0.5 ± 0.0^{17}	pu	3.2 ± 1.1^{5}	0.3 ± 0.0^3	1.1 ± 0.1^8
Neolissochilus hexagonolepis	213.7 ± 16.0^{19}	574.5 ± 32.8^{25}	1175 ± 27.6^{19}	nd	nd	$2\pm0.0^{5.6}$	nd	1.9 ± 0.0^{17}	0.2 ± 0.0^1	1.7 ± 0.0^9
Oncorhynchus mykiss	215.2 ± 12.12^{19}	1217.4 ± 96.3^{26}	418.6 ± 99.1^{20}	pu	nd	$1.3\pm 0.9^{14,16}$	nd	1.3 ± 0.4^{2}	0.2 ± 0.0^1	0.6 ± 0.2^7
Schizothorax richardsonii	226.8 ± 99.2^{25}	1158.1 ± 99.8^{27}	414.7 ± 99.1^{21}	pu	nd	0.6 ± 0.0^{17}	nd	2.2 ± 0.5^4	0.3 ± 0.0^3	0.6 ± 0.0^7
Tor putitora	172.5 ± 19.9^{26}	1016.1 ± 98.2^{28}	116 ± 70.6^{22}	nd	nd	$0.9\pm 0.1^{16,17}$	pu	1.3 ± 0.0^2	0.2 ± 0.0^1	0.9 ± 0.2^{11}
Brackishwater										
Lates calcarifer	264.4 ± 3.0^{27}	1086.3 ± 19.6^{29}	241.9 ± 4.3^{23}	149.8 ± 2.2^8	920.9 ± 5.0^{15}	11.8 ± 0.3^{13}	0.5 ± 0.0^6	5.56 ± 0.6^{19}	0.2 ± 0.0^1	0.2 ± 0.0^2

Fish species	Macromineral					Micromineral				
	Na	K	Ca	Mg	Ρ	Fe	Cu	Zn	Mn	Se
Mugil cephalus	202.3 ± 8.2^{17}	878.3 ± 38.7^{30}	$263.9 \pm 6.7^{24} \qquad 142.0 \pm 3.2^9$	142.0 ± 3.2^{9}	$892.3 \pm 14.5^{16} 12.8 \pm 0.6^{13} 1.4 \pm 0.1^5 5.1 \pm 0.0^{18}$	12.8 ± 0.6^{13}	1.4 ± 0.1^{5}	5.1 ± 0.0^{18}	0.04 ± 0.0^2 0.1 ± 0.0^1	0.1 ± 0.0^1
Prawn Pendeus monodon	831+43 ²⁸	$1733 \ 7 + 20 \ \xi^{31}$	$410 3 + 3 2^{20}$	$281 7 + 4 04^{10}$	$2817 + 4.04^{10} 1970 3 + 76.0^{17} 16.41 + 0.9^{18} 4.18 + 0.06^7$	16.41 + 0.9 ¹⁸	4.18 ± 0.06^7	$7 34 \pm 0.04^4$	0.31 ± 0.0^{3}	0 13 + 0 0 ¹
Fenneropenaeus indicus	809 ± 10.1^{29}	1041.68 ± 20.8^{32}	429.7 ± 7.4^{25}	252.0 ± 10.0^{11}	252.0 ± 10.0^{11} 1248 ± 50.7^{18}	10.81 ± 0.3^9	4.36 ± 0.4^{8}	6.09 ± 0.8^{21}	0.21 ± 0.0^{1}	0.11 ± 0.01^{1}
Mollusc										
Crassostrea madrasensis	166.3 ± 26.4^{11}	390.4 ± 89.7^{33}	145 ± 25.7^2	nd	422 ± 91.8^{19}	3.7 ± 0.5^7	nd	$0.43\pm 0.0^{16,20} 3.9\pm 0.4^{11}$	3.9 ± 0.4^{11}	0.03 ± 0.0^3
Perna viridis	1810.2 ± 91.4^{30}	$1810.2 \pm 91.4^{30} 1310.9 \pm 92.3^{34}$	522.6 ± 38.7^{26} 251 ± 22.1^{12}	251 ± 22.1^{12}	pu	6.3 ± 2.6^{10}	0.2 ± 0.1^3	$2.59 \pm 0.7^{4.6} \qquad 0.6 \pm 0.3^{12}$	0.6 ± 0.3^{12}	0.04 ± 0.0^5
P value*	3.796E-205	5.62E-111	3.5E-120	6.74E-96	7.37E-125	1.18E-51	2.5522E-47	2.5522E-47 4.83107E-57 3.416E-55 6.70E-46	3.416E-55	6.70E-46
The values are expressed as mg/100 g wet weight. Values are reported as mean ± standard deviation of 16 individual fish samples analyzed in triplicate. For the SIFs, prawns, and mollusc, six pooled samples (each containing 100 of individual samples) were analyzed in triplicates. Values in columns not sharing same superscripts differ significantly at 0.05 level of significance	100 g wet weight. V individual samples)	Values are reported a were analyzed in the	as mean±standaı riplicates. Values	rd deviation of 16 in columns not sl	5 individual fish sa haring same supers	mples analyzed cripts differ sign	in triplicate. Fo nificantly at 0.05	or the SIFs, prawn 5 level of signific	ns, and mollus ance	c, six pooled

Table 4 (continued)

100 g) followed by *Stolephorus commersonii* (21.0 mg/100 g) and freshwater garfish *Xenontodon cancila* (21.3 mg/100 g).

Copper is required for iron utilization and as a cofactor for enzymes involved in glucose metabolism and the synthesis of hemoglobin, connective tissue, and phospholipids [30]. The copper content ranged from 0.2 to 4.36 mg/100 g, significantly higher (P < 0.05) in prawns *Fenneropenaeus indicus* (4.36 mg/100 g) and *Penaeus monodon* (4.18 mg/100 g) far exceeding that in fish species which is similar to an earlier report [31] and is higher than results reported elsewhere [25].

Manganese is a trace mineral that is present in the human body in very small amounts, primarily in the bones, liver, kidneys, and pancreas. It is important in the formation of the bones, connective tissues, blood clotting factors, and sex hormones and also is involved in fat and carbohydrate metabolism, calcium absorption, and blood sugar regulation. In addition, it is important for brain and nerve function. The SIF *Gudusia chapra* (4.61 mg/100 g) were found to be very rich in manganese at P < 0.05 followed by *Xenontodon cancila* (1.47 mg/100 g), *Amblypharyngodon mola* (1.1 mg/100 g), and *Puntius sophore* (1.1 mg/100 g).

Selenium is an essential trace mineral of fundamental importance to human health. As a constituent of selenoproteins, selenium has structural and enzymic roles, in the latter context being best known as an antioxidant and catalyst for the production of active thyroid hormone [32]. The selenium content of the fishes and shellfishes showed a wide range from 0.03 to 1.7 mg/100 g, significantly higher (P < 0.05) in coldwater fish *Neolissochilus hexagonolepis* than the other fish species studied.

Fat-Soluble Vitamins

 *P value was considered to be significant at 0.05 level of significance

nd not determined

Vitamins are a group of substances that are essential for normal cell function, growth, and development. There are four fatsoluble vitamins namely vitamin A, D, E, and K. Each of the vitamins has important functions in the body, and a vitamin deficiency can cause many health problems. Vitamin A is required for normal vision and for growth of the bones. Vitamin A derivative retinoic acid regulates gene expression in the development of epithelial tissue. It also plays crucial role in normal immune function. Vitamin A has been found to be playing a major role in combating diseases like malaria [33]. An estimated 250 million preschool children are vitamin A deficient, and it is likely that in vitamin A-deficient areas, a substantial proportion of pregnant women are vitamin A deficient [34]. The freshwater SIF Amblypharyngodon mola (554.9 IU/100 g) contains significantly higher (P < 0.05) amount of vitamin A followed by marine fishes Epinephelus spp. (379.3 IU/100 g) and Sardinella longiceps (346.4 IU/100 g). Amblypharyngodon mola have been previously reported to play a potential role in food-based strategies to address vitamin A deficiencies in Bangladesh [35]. The migratory fish Tenualosa ilisha (260.7 IU/100 g) and SIFAnabas testudineus (89.8 IU/100 g) were also very rich in vitamin A. The

vitamin A content of these fishes was found to be higher than 20 different fish species including mackerel, salmon, dogfish [36], rainbow trout (74.33 IU/100 g) [37], common carp (75.06 IU/ 100 g), and European catfish (21.0 IU/100 g) [38]. Similarly, the vitamin D content was highest in the SIF Amblypharyngodon mola (9312.4 IU/100 g) followed by Puntius sophore (16, 266.4 IU/100 g) at P < 0.05 [26], Tenualosa ilisha (9549 IU/ 100 g), and marine fishes Epinephelus spp. (23,445.9 IU/100 g). Vitamin D is required to maintain normal blood levels of calcium and phosphate that are in turn needed for the normal mineralization of the bone, muscle contraction, nerve conduction, and general cellular function in all cells of the body. Vitamin D possesses curative properties for chronic diseases like osteoporosis, cancer, cardiovascular diseases, and diabetes [39, 40]. Vitamin D present in fish liver and oils is crucial for bone growth since it is essential for absorption and metabolism. Recent studies have found that lower serum vitamin D concentration is associated with depression and mood disorders [41].

Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defense system and is exclusively obtained from the diet. The major biologic role of vitamin E is to protect polyunsaturated fatty acids and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals [28, 42]. Besides, it plays many important physiological roles in enzymatic reactions [43], regulation of gene expression, and growth inhibition of cancer [44]. The vitamin E content of marine fishes, *Epinephelus* spp. and *Sardinella longiceps*, was significantly higher (P < 0.05), i.e., 108.0 and 98.2 IU/100 g, respectively, than *Tenualosa ilisha* (97.7 IU/100 g) which is also much higher than common carp (0.46 mg/100 g), pike perch (0.94 mg/100 g), and European catfish (0.80 mg/100 g) [38].

Vitamin K acts as an essential cofactor for carboxylation of certain glutamate residue to γ -carboxy-glutamate residue in a selected number of proteins. The majority of known γ -carboxy-glutamates containing or vitamin K-dependent proteins are involved in blood coagulation. The SIFs *Amblypharyngodon mola* (4092.2 IU/100 g) followed by *Puntius sophore* (884.2 IU/100 g) and marine fish *Epinephelus* spp. (789.6 IU/100 g) were found to be significantly high in vitamin K at *P* < 0.05 than the other fish species (Table 5).

Table 5 Fat-soluble vitamin content of important food fishes from India

Fish species	Vitamin A (IU/ 100 g wet weight)	Vitamin D (IU/ 100 g wet weight)	Vitamin E (IU/ 100 g wet weight)	Vitamin K (µg/ 100 g wet weight)
Marine				
Epinephelus spp.	379.3 ± 25.2^{1}	$23,445.9 \pm 52.3^{1}$	108.0 ± 45.7^{1}	789.6 ± 56.2^{1}
Katsuwonus pelamis	3.3 ± 1.2^{2}	435.5 ± 45.3^2	$0.6 \pm 0.1^{2,3}$	$0.9 \pm 0.1^{2,3}$
Leiognathus splendens	2.9 ± 1.2^{2}	597.6 ± 21.2^3	$0.7 \pm 0.1^{2,3}$	$1.1 \pm 0.2^{2,3}$
Sardinella longiceps	346.4 ± 25.2^3	$21,\!288.0\pm25.3^4$	98.2 ± 24.0^4	717.8 ± 26.3^4
Trichiurus lepturus	2.9 ± 1.3^{2}	316.3 ± 13.2^5	$0.5 \pm 0.2^{2,3}$	1.2 ± 0.3^{3}
Freshwater				
Amblypharyngodon mola	555.0 ± 56.2^4	$93,122.4\pm 89.2^{6}$	2.2 ± 0.1^{5}	4092.2 ± 65.2^{5}
Anabas testudineus	89.8 ± 15.2^5	64.4 ± 23.2^7	0.9 ± 0.1^{3}	$0.8 \pm 0.2^{2,3}$
Catla catla	12.2 ± 4.5^{6}	59.5 ± 12.5^{8}	$0.5 \pm 0.1^{2,3}$	$0.5 \pm 0.1^{2,3}$
Cirrhinus mrigala	30.9 ± 8.9^{7}	152.3 ± 25.3^9	$0.6 \pm 0.2^{2,3}$	0.2 ± 0.0^2
Clarias batrachus	$3.9 \pm 1.1^{2,7}$	30.9 ± 10.2^{10}	$0.3 \pm 0.1^{2,3}$	$0.4 \pm 0.1^{2,3}$
Heteropneustes fossilis	26.8 ± 9.8^8	111.7 ± 14.2^{11}	$0.2 \pm 0.0^{2,3}$	2.7 ± 0.3^{6}
Labeo rohita	8.7 ± 2.2^{9}	84.4 ± 11.2^{12}	$0.6 \pm 0.2^{2,3}$	$0.7 \pm 0.1^{2,3}$
Puntius sophore	70.9 ± 12.3^{10}	$16,266.4 \pm 84.2^{13}$	3.4 ± 0.3^6	884.2 ± 24.2^7
Sperata seenghala	56.2 ± 15.2^{11}	7494.8 ± 56.3^{14}	0.1 ± 0.0^2	166.7 ± 26.3^8
Tenualosa ilisha	260.7 ± 45.2^{12}	9549 ± 85.2^{15}	97.7 ± 12.6^7	203.3 ± 26.3^9
Mollusc				
Crassostrea madrasensis	4.6 ± 2.2^{13}	451.6 ± 12.3^{16}	$0.2\pm 0.0^{2,3}$	1.2 ± 0.2^{3}
Perna viridis	9.5 ± 4.5^{9}	404.3 ± 11.2^{17}	0.1 ± 0.0^2	2.2 ± 0.1^{6}
P value	2.02054E-88	6.5246E-160	1.21357E-74	1.1431E-117

Values are reported as mean \pm standard deviation of 16 individual fish samples analyzed in triplicate. For the mollusc, six pooled samples (each containing 100 of individual samples) were analyzed in triplicates. Values in columns not sharing same superscripts differ significantly at 0.05 level of significance. The amount microgram per kilogram was converted to IU by online vitamin converter. Conversion factors: vitamin A 1 IU = 0.3 µg, vitamin D 1 IU = 0.025 µg, and vitamin E 1 IU = 671 µg http://www.robert-forbes.com/vitamin-converter

*P value was considered to be significant at 0.05 level of significance

Micronutrients	Species rich in particular micronutrient
Macromineral	
Sodium	Perna viridis, Penaeus monodon, Fenneropenaeus indicus
Potassium	Rastrelliger kanagurta, Stolephorus commersonii, Nemipterus japonicus
Calcium	Xenentodon cancila, Gudusia chapra, Ailia coila, Neolissochilus hexagonolepis, Puntius sophore
Magnesium	Penaeus monodon, Fenneropenaeus indicus, Perna viridis
Phosphorous	Xenentodon cancila, Gudusia chapra, Epinephelus spp.
Micromineral	
Iron	Gudusia chapra, Amblypharyngodon mola, Puntius sophore
Copper	Thunnus albacares, Xenentodon cancila
Zinc	Stolephorus waitei, Xenentodon cancila, Stolephorus commersonii
Manganese	Gudusia chapra, Crassostrea madrasensis, Xenentodon cancila
Selenium	Neolissochilus hexagonolepis, Labeo rohita, Clarias batrachus
Fat-soluble vitamins	
Vitamin A	Amblypharyngodon mola, Epinephelus spp., Sardinella longiceps
Vitamin D	Amblypharyngodon mola, Puntius sophore, Epinephelus spp.
Vitamin E	Epinephelus spp., Sardinella longiceps, Tenualosa ilisha
Vitamin K	Amblypharyngodon mola, Puntius sophore, Epinephelus spp.

 Table 6
 Fish rich in specific minerals and fat-soluble vitamins among the species studied are listed

Ref. Tables 4 and 5. The recommendations are according to RDA [20], which is depicted in Fig. 1

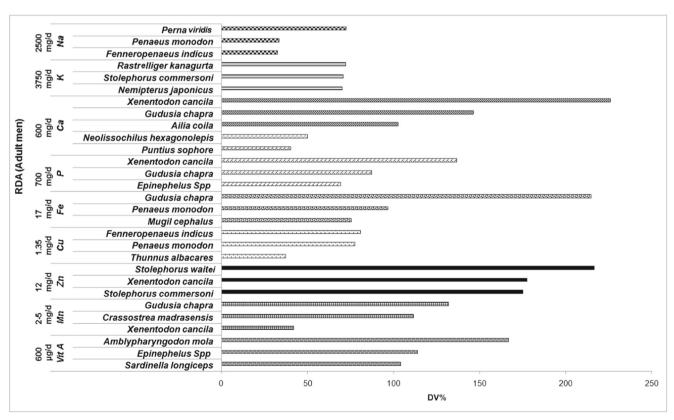


Fig. 1 Daily value (DV %) of one serving of fishes and shellfishes (Table 6) for important micronutrients. The RDA recommended for adult men is provided along the *Y*-axis, and accordingly, the potential contribution of fish and shellfishes (DV %) is plotted along the *X*-axis. DV% was calculated using the analyzed values and recommended daily

allowance (RDA) recommended for adult men weighing 60 kg for Indians (ICMR 2010) with appraisal of FAO/WHO recommendations. For calculation of DV%, each serving was considered to be 100 g of fish or shellfish (except for Ca, Cu, and P for which it was 25 g)

Consumption of Fishes and Shellfishes in Relation to RDA of Nutrients

The RDA projects the quantitative recommendation of nutrients for people in general to stay healthy. This may be different for different categories like children, adult males, and females. The Indian Council for Medical Research (ICMR) favors the FAO/WHO/UNU guidelines for framing the RDA guidelines, with slight modifications. The RDA for a nutrient will be in a standard unit which helps the common man to easily calculate his own requirements based on the body weight and/or basal metabolic rate. The potential contributions of fishes and shellfish species (DV %) to RDA (for an adult man weighing 60 kg) of important micronutrients are given in Fig. 1. These values are calculated for raw fish; however, dietary bioavailability is dependent on a number of factors. Ca, Mg, Cu, I, and Se are relatively well absorbed, with reported fractional absorption values from mixed diets in man, and less well-absorbed trace elements include Fe, Zn, Mn, and Cr, with absorption varying widely according to the nutritional status (including body stores) of the individual and the composition of the diet [45]. The richness of fish and shellfishes in this study for specific micronutrients (Table 6) is based on its potential contribution (DV %) to the RDA for that nutrient; for example, the green mussel Perna viridis which was found to very rich in sodium contributes 72.41 %(50-g serving) of the daily sodium requirement. Similarly, the SIF Amblypharyngodon mola which is rich in vitamin A contributes 83.25 % (50-g serving) of the daily vitamin A requirement (Table 6, Fig. 1).

Conclusion

Fish is an important source of micronutrients, and in comparison to the other sources of micronutrients, the consumers have a wide choice for fish, as there are many varieties and species of fishes available, especially in the tropical countries [13]. However, the nutrient composition of fish varies with species, their habitat, feeding behavior, and many other factors. Therefore, we have generated information on the mineral and vitamin composition of 35 Indian food fishes which has enriched the nutrient composition knowledgebase. This information would increase the utility of these fishes in community nutrition by creating awareness among the consumers about their nutritional importance. The information generated would also be useful in clinical nutrition by providing a reference point to clinicians and dieticians to prescribe specific fish for specific clinical requirement. Based on our study, in general, the shellfishes and marine fishes can be recommended for macrominerals sodium and potassium; SIFs for calcium, iron, and manganese; coldwater fishes for selenium; and the brackishwater fishes for phosphorous. The marine fishes especially *Sardinella longiceps* and *Epinephelus* spp. were found to be rich sources of fat-soluble vitamins A, D, E, and K, followed by SIFs *Amblypharyngodon mola* and *Puntius sophore*. All the recommendations are based on the potential contribution of the fish and shellfishes to the RDA for that nutrient. Information on the micronutrients generated would enhance the utility of fish in both community and clinical nutrition.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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