Research Article

Analysis of Trace and Macroelements of *Lepidocephalichthys berdmorei* in Relation to Sex and Seasonal Variation

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ABSTRACT

Minerals are essential in determining the overall health status of different organisms. Fishes can directly absorb and retain minerals from the water through their gill or skin. The amount of minerals in fish depends on the habitat and the diet the fish consumes. The present study determines the content of trace elements and macro elements in both sexes of *Lepidocephalichthys berdmorei*, a hill stream loach found in Manipur, India, for three seasons using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). The concentration of 8 trace elements (Fe, Zn, Mn, Cu, Co, Ni, Cr and Se) and 5 essentials macro elements (Ca, P, K, Na and Mg) have been analyzed for both sexes. Male species had various elements with concentrations ranging from 4895.95±5.01 mg/100 g (Ca) to 0.0113±0.0023 mg/100 g (Co). Females had concentrations ranging from 3712.52±9.08 mg/100 g (Ca) to 0.013±0.00 mg/100 g (Co). Calcium, Phosphorus and Potassium were the most abundant macro elements in males, whose peak concentrations were during the post-monsoon season. In females, Ca and P were abundant, with different seasonal concentrations. Among the trace elements, Fe and Zn had the highest concentration, 8.03±0.32 mg/100 g (Fe) and 4.62±0.91 mg/100 g (Zn) in males and in females, 11.72±0.41 mg/100 g (Fe) and 5.42±0.33 mg/100 g (Zn) respectively. Ni and Se were detected in very low concentrations, below 0.01 ppm. Mineral content in aquatic habitats differs in different seasons; accordingly, the values of the minerals differ in seasons and both sexes. The analysis revealed the importance and role of minerals in the growth of the fish, which would help in the sustainable and judicious management and conservation of this fish and could be helpful to nutritionists, researchers, fish biologists, conservationists, farmers, etc., for future reference.

Keywords: ICP-AES, *Lepidocephalichthys berdmorei*, Macro elements, Trace elements.

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INTRODUCTION

Minerals are essential for their vital physiological and biochemical functions and maintenance of their life processes and in determining the overall health status of different organisms. Fishes can directly absorb and retain minerals from the water through their gill or skin.[1] The amount of minerals present in fish depends on the habitat and the type of diet the fish consumes.[2] The essentiality of macro minerals (calcium, phosphorus, magnesium, sodium, potassium) and trace elements (cobalt, copper, chromium,nickel, iron, manganese, selenium, zinc) in animals and other vertebrates, including fish, have been confirmed. [1] However, in fishes, trace elements such as Cr and Ni are considered essential for humans and animals based on the impairment of specific physiological functions, which have not been reported in previous studies. Generally, fish and terrestrial animals have similar biochemical mechanisms of mineral metabolism at the cellular level. Essential macro- and trace minerals at optimal levels are required to grow and maintain fish's

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overall health. Trace elements are widely recognized for their catalytic, structural, physiological, regulatory and metabolic functions.[3] Over one-third of all proteins require a trace element cofactor for normal function[4,5] such as energy production, protein digestion, cell replication and antioxidant activity. Trace element in deficient or suboptimum levels may cause a decrease in or loss of enzyme activities.[6]

Lepidocephalichthys berdmorei (known as ngakijou in Manipuri) is a small loach with sexually dimorphic pectoral fins distributed throughout south and southeast Asia, including India, Myanmar and Thailand. There are 18 species under Genus *Lepidocephalichthys* under the family Cobitidae. In mature males, the pectoral fins have been enlarged with fused, thickened innermost seventh and eighth rays, forming a structure known as the lamina circularis.[7] This structure is absent in females and indistinguishable in juveniles. This is present in most other cobitid genera, though usually formed by the second ray.[7] Adult females are typically heavier-bodied and larger than males. This loach has an omnivorous diet and nocturnal behaviors and prefers fine gravel and sand as habitat. It is sensitive to light, so it prefers nocturnal habitats. The knowledge in the area of nutrient variability based on sex and season is scant compared to other animals and humans. This study was designed to determine the concentrations of different elements in *Lepidocephalichthys berdmorei* in pre-monsoon, monsoon and post-monsoon seasons in both sexes using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

MATERIALS AND METHODS

Collection of samples

The samples were collected from the sampling sites- Moirang (24.501845, 93.768159) and Serou (24.254612, 93.887246) Manipur during the pre-monsoon, monsoon and post-monsoon seasons from March 2021 to September 2022. The male and female fish samples were identified and their length and wet weight were measured. Similar-sized fishes were used for the analysis. The average length ranged within 8-9 cm and the weight ranged from 3.68-5.12 g for both sexes, respectively. These were dried in a hot air oven at 60ºC till a constant weight was obtained, after which they were ground into fine powder.

Sample preparation

Approx. 0.1 g sample was weighed and 4 mL conc. HNO_3 was added. The samples were subjected to microwave digestion using Anton Paar Microwave Go. The heating temperature was set at 190ºC (Ramp-15 min; Hold-25 min). The samples were cooled to room temperature and then made up to 15 mL with distilled water.

Instrumentation

The analysis was conducted at the ICP-AES laboratory in SAIF IIT Bombay CRNTS facility using ARCOS, Simultaneous ICP Spectrometer (SPECTRO Analytical Instruments GmbH, Germany). The Spectrometer has a 130 to 770 nm wavelength range and a resolution of approx. 9 pm. The system has Charge Coupled Devices (CCD), Nebulizers and Spray chambers. The wavelengths used for detecting the selected elements are given in Table 1.

Table 1: Wavelengths (in nm) used for the selected elements.

Element	Wavelengths used (nm)
Ca	422.673
Mg	279.079
Na	589.592
K	766.491
P	213.618
Co	228.616
Cr	267.716
Cu	324.754
Fe	259.941
Mn	259.739
Ni	231.604
Zn	213.856
Se	196.09

Statistical Analysis

Experiments were done in triplicates. The statistical analysis was done using IBM SPSS Statistics Version 21 and the results were expressed as mean±SD. One-way Analysis of Variance (ANOVA) was performed and the mean values of both sexes in all seasons were compared using Tukey *post hoc* tests at a 5% significance level. Correlation was considered significant when $p<0.05$ and $p<0.01$ were obtained.

Ethics approval

The work was carried out as the Institutional Animal Ethics Committee (IAEC) of Manipur University approved the work.

RESULTS

Altogether, five macro elements (Ca, P, K, Na and Mg) and eight trace elements (Fe, Zn, Mn, Cu, Cr, Co, Ni and Se) were analyzed in *Lepidocephalichthys berdmorei*. In the present study, the elemental content was found in varying concentrations among the sexes and during the seasons. Overall, the value of macro elements in males is higher than that of females. Also, in both males and females, the magnitude of concentration decreases in the order of Ca> P> K> Na> Mg. However, in most of the trace elements, females had a higher concentration than males. The concentration of trace elements in decreasing order in both males and females is as follows: Fe>Zn>Mn>Cu>Cr>Co>Ni>Se. However, Co was not detected in males during monsoon and post-monsoon seasons, and in females during pre-monsoon season. Ni and Se were not detected at all, which means these are present in quantities below 0.01 ppm since the instrument sensitivity is more than 0.01 ppm. The comparison of the mean values using Tukey's *post hoc* test revealed significant differences ($p<0.05$) in the values of the concentrations in the different seasons.

Tables 2 and 3 give macro and trace element values, respectively. Although the values given by the instrument is in ppm, they are converted into mg/100 g for our convenience and for better comparisons with other references.

The overall relationships of all the macro and trace elements were calculated. Table 4 gives the correlation values of the elements. Ca is positively correlated with Mg, P and K with high correlation values of 0.907, 0.994 and 0.823, respectively and negatively correlated with Co, Cu and Fe with high correlation values of -0.870, -0.775 and -0.719, respectively at $p\leq0.01$ level. Mg is positively correlated with *p* (0.913) and K (0.784) and negatively correlated with Co (-0.810), Cu (-0.664) and Fe (-0.715) at $p\leq0.01$ level. Na is negatively correlated with Mn (-0.793) and Zn (-0.827) at $p\leq0.01$ level. P is positively correlated with K (0.780) and negatively correlated with Co (-0.852), Cu (-0.748) and Fe (-0.735) at $p\leq0.01$ level. K is negatively correlated with Co (-0.891), Cu (-0.741) and Fe (-.0814) at $p\leq0.01$ level. Co is positively correlated with Cu and Fe with high correlation coefficients of 0.939 and 0.851, respectively, at $p\leq0.01$ level. Cu is positively correlated with Fe (0.660) and negatively correlated with Mn (-0.607) at $p\leq0.01$ level. The values within the brackets indicate the correlation coefficients of each of the elements.

At $p\leq0.05$ level, Mg positively correlates with Na (0.506) and negatively correlates with Zn (-0.480). Co negatively correlates with Mn (-0.541) at $p\leq0.05$ level. Fe is negatively correlated with Mn (-0.487) at $p\leq0.05$

Table 2: Macro element composition of *Lepidocephalichthys berdmorei* analysed by ICP-AES.

	Male			Female		
	Pre-monsoon season	Monsoon season	Post-monsoon season	Pre-monsoon season	Monsoon season	Post-monsoon season
Ca	4726.20±4.01^e	4196.60±4.01^d	4895.95±5.01^f	3712.52±9.08^c	3026.29±4.63^b	2518.28±11.07^a
P	2883.13±4.01^e	2596.49±4.01^d	2918.84±4.01^f	2427.31±5.80^c	2249.21±9.94^b	1970.27±10.99^a
K	938.99±2.01^e	964.28±2.00^f	894.20±2.00^c	906.29±4.73^d	802.54±5.72^b	783.35±4.51^a
Na	260.94±2.01^c	339.87±2.01^e	256.98±2.01^c	152.12±4.75^a	279.70±7.92^d	238.01±6.66^b
Mg	194.74±2.01^c	197.19±2.01^c	199.27±2.04^c	166.97±4.70^b	172.12±6.64^b	145.33±5.76^a

Values are given in mg/100 g, Mean±SD (*n*=3). Values in the same row with the same superscripts are not significantly different (Significance $p< 0.05$).

Table 3: Trace element composition of *Lepidocephalichthys berdmorei* analysed by ICP-AES.

	Male			Female		
	Pre-monsoon season	Monsoon season	Post-monsoon season	Pre-monsoon season	Monsoon season	Post-monsoon season
Fe	5.244±0.025^a	8.150±0.020^b	10.692±0.035^e	8.362±0.026^c	10.396±0.025^d	16.424±0.045^f
Zn	5.457±0.040^d	3.654±0.035^a	4.784±0.035^b	5.794±0.045^e	5.259±0.030^c	5.204±0.035^c
Mn	1.423±0.025^d	1.006±0.015^b	1.226±0.05^c	1.993±0.035^e	0.996±0.002^b	0.9444±0.015^a
Cu	0.3785±0.0103^c	0.3505±0.0093^b	0.309±0.0101^a	0.3112±0.0102^a	0.4204±0.010^d	0.4998±0.010^e
Cr	0.081±0.002^a	0.179±0.010^d	0.1312±0.0102^c	0.1691±0.0086^d	0.2308±0.0112^e	0.1116±0.0125^b
Co	0.0113±0.0023^a	ND	ND	ND	0.1393±0.011^b	0.2790±0.0101^c
Ni	ND	ND	ND	ND	ND	ND
Se	ND	ND	ND	ND	ND	ND

Values are given in mg/100 g and ND: Not detected <0.01 ppm; Mean±SD (*n*=3)
Values in the same row with the same superscripts are not significantly different. (Significance $p< 0.05$).

Table 4: Pearson's correlation among the different elements.

	Ca	Mg	Na	P	K	Co	Cr	Cu	Fe	Mn	Zn
Ca	1										
Mg	.907**	1									
Na	.184	.506*	1								
P	.994**	.913**	.192	1							
K	.823**	.784**	.206	.780**	1						
Co	-.870**	-.810**	-.028	-.852**	-.891**	1					
Cr	-.344	-.081	.160	-.329	-.199	-.019	1				
Cu	-.775**	-.664**	.156	-.748**	-.741**	.939**	-.110	1			
Fe	-.719**	-.715**	-.100	-.735**	-.814**	.851**	.014	.660**	1		
Mn	.295	.017	-.793**	.276	.394	-.541*	-.124	-.607**	-.487*	1	
Zn	-.250	-.480*	-.827**	-.200	-.394	.202	-.193	.132	.021	.581*	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

level. At $p \leq 0.05$ level, Mn is positively correlated with Zn (0.581).

DISCUSSION

The variations in the concentrations may be related to various factors such as diet and habitat. However, it does not mean that the fish will readily utilize minerals from water. Seasonal variations were observed for specific elements and higher levels were observed during the post-monsoon season in males. Also, due to the non-availability and scarcity of the samples during the winter season (Nov-Jan), the values of the minerals during this period are missed.

Fish require minerals to reproduce as minerals take part in various important biochemical processes.[2] Most researchers observed no definite order in the magnitude of the elements as their variations in concentration may be due to the chemical forms of the elements and their concentration in the environment.[8] Fish are good providers of nutrients, but the variations that have been noted may be caused by the fishes' poor capacity for consuming, absorbing and processing vital nutrients from their diet or environment into the biochemical qualities they require.[9]

The values of the elements, as detected and quantified by ICP-AES, are much more sensitive and accurate than SEM EDX. However, values detected by the EDX also give results almost in agreement with those obtained by ICP-AES (Unpublished data).

Among all the elements, Ca has the highest concentration. In males, the concentration of Ca is highest in post-monsoon season, followed by pre-monsoon season and monsoon season. This must be because of high activity of male engaged in reproduction during monsoon season. In post-monsoon season, the overall activity of males is reduced and hence, the value of Ca increases. In females, Ca concentration is highest in the pre-monsoon season, which is followed by the monsoon season and post-monsoon season. This gradual decline in the Ca concentration might be due to egg formation and subsequent discharge of the eggs which might cause a decrease in post-monsoon ranges. The value of Ca in this species is higher as compared to other reports of fish belonging to the same family[10] which may be because of the sensitivity of the instrument. However, the value is higher in *L berdmorei* than in *A. coila* (2410±14.7 mg/100 g), as studied by Mohanty *et al.*,[11] which was done using ICP-AES. The value is almost in agreement with *G. chapra* (3440±10.4 mg/100 g).[11] The present investigation indicated the presence of the highest level of Ca among other studied macro elements during all three seasons, which is in agreement with Shantosh and Sarojnalini[10] for *Lepidocephalichthys guntea, Pangio pangia and Syncrossus berdmorei*, Mayanglambam S and Chungkham S[12] for *Devario yuensis*, *Glossogobius giuris*, *Hypsibarbus myitkyinae*, *Puntius chola* and *Tariqilabeo burmanicus* and Sharma S and Singh D[13] for *Schizothorax richardsonii*. The body requires more abundant levels of macro elements for its structure and function, which was also noticed by Hei and Sarojnalini.[14] The present findings indicate that the values are higher than previous findings as this was done using a more sophisticated and sensitive instrument than the previous ones.

The ratio of Ca and P, which is between 1.6:1 or 2:1 in almost all other studies, also proved that the values obtained in the study are almost in good agreement, with ratios ranging from 1.62:1 to 1.68:1 in males, however with slightly less ratios ranging from 1.30:1 to 1.53:1 in females. P concentration in males and females is similar to the order of Ca. P is necessary for fish reproduction, energy metabolism, optimum growth, development and maintenance of the skeletal system.[15] In the present study, the content of P is higher than that of the concentration reported by the previous workers, such as in *Anabas testudineus* (159.8±3.5 mg/100 g), *Ailia coila*, (1880±45.2 mg/100 g), *Gudusia chapra* (2490±32.1 mg/100 g) which is analyzed with AAS and ICP-AES.[11] Likewise, Na and K concentrations are much higher than in the previous report by Shantosh and Sarojnalini.[10] Their report showed the content of Na in the Small Indigenous Fishes in the range of 45.0±0.20 mg/100 g to 112.5±0.06 mg/100 g and K in the range of 57.5±10.15 mg/100 g to 90.81±1.41 mg/100 g. In the present study, the concentration of K in monsoon season is highest (964.28±2.00 mg/100 g), followed by pre-monsoon season (938.99±2.01 mg/100 g) and post-monsoon season (894.20±2.00 mg/100 g) in males. In females, the highest concentration of K is in pre-monsoon season (906.29±4.73 mg/100 g), followed by monsoon (802.54±5.72 mg/100 g) and post-monsoon (783.35±4.51 mg/100 g) seasons. The value of K ranged from 90.81±1.41 mg/100 g in *S berdmorei*, 87.33±0.29 mg/100 mg in *L guntea and* 57.5±0.15 mg/100 g in *Pangia*.[10] Also the values varies between 149.8±1.14 mg/100 g to 47.49±0.10 mg/100 g in *Glossogobius giuris* and *Hypsibarbus myikyinae*.[12] The concentration of Na is highest in the monsoon season in both males (339.87±2.01 mg/100g) and females (279.70±7.92 mg/100g), followed by pre-monsoon season and post-monsoon season in males and vice versa in females. The concentration of Mg is highest in the post-monsoon season (199.27±2.04 mg/100 g), followed by the monsoon season (197.19±2.01 mg/100 g) and pre-monsoon season (194.74±2.01 mg/100 g) in males. In females, the highest concentration is found in the monsoon season (172.12±6.64 mg/100 g), followed by pre-monsoon season (166.97±4.70 mg/100 g) and post-monsoon season (145.33±5.76 mg/100 g). The concentration of Mg in *Ailia coila* is 160.0±12.3 mg/100 g, *Gudusia chapra* (170±9.8 mg/100 g) (Mohanty P *et al.*),[11] *Lepidocephalichthys guntea* is 87.33±0.29 mg/100 g (Shantosh *et al.*),[10] respectively. The present study has higher values compared to previous studies.

The concentration of Fe ranges from 16.424±0.045 mg/100 g to 8.362±0.026 mg/100 g in females while in males, the range is from 10.692±0.035 mg/100 g to 5.244±0.025 mg/100 g. Fe is one of the most investigated essential trace elements that is found in all body cells of vertebrates. It plays a crucial role in various biochemical processes, such as the electron transfer reaction, gene regulation, cell growth and differentiation regulation, oxygen binding and transport and gene regulation.[16] The result was in agreement with those of the following studies: Jithesh and Radhakrishnan[17] for *Diplodus annularis*, Khitouni *et al*.[18] for *Trichiurus lepturus,* Danabasa *et al*.[19] for *Barbus* sp. and *Cyprinus carpio* and Njinkoue *et al*.[20] for *Pseudotolithus typus* and *Pseudotolithus elongatus*. The values are also in agreement with *Ailia coila* (10.9±1.3 mg/100 g), *Amblypharyngodon mola* (11.9±3.4 mg/100 g), *Puntius sophore* (11.6±3.6 mg/100 g).[11] The research of Cross *et al*.,[21] Hei and Sarojnalini,[14] Kumar *et al*.,[22] Durmus *et al*.[23] and Sarma *et al*.[24] also supported the current findings of higher Fe values followed by Zn. Compared to the studies by Rahman *et al*.[25] and Romharsha and Sarojnalini,[26] the concentration of Zn and Cu was found to be much greater in the current study. The present study has concentrations higher than *Anabas testudineus* (0.9±1.0 mg/100 g), *Amblypharyngodon mola* (3.9±1.3 mg/100 g), *Puntius sophore* (5.4±0.4 mg/100 g) According to Sivaperumal *et al*.,[27] copper is necessary for fish development, reproduction and synthesis of hemoglobin and it is a necessary component of several enzymes. Fish muscle contained more Zn and Cu metals in the summer than it did during the monsoon, possibly because fish respire more quickly in the summer. This is also supported in our study as the values are higher in the pre-monsoon season as compared to the monsoon season. The concentration of dissolved oxygen in the aquatic environment is generally lowered at higher temperatures, which accelerates the metabolic digestion of fish. When a fish grazes on grasses and plants, it needs to drink more water, which raises the quantity of metals it absorbs actively or by diffusion. Conversely, fish that are less active during other seasons have lower metal concentrations (Zayed *et al.,*).[28] Hei A, 2013 reported that the concentration of copper in hill stream fish was in the range of 0.299 mg/100 g to 1.50 mg/100 g.[29] Also, Cu was not detected in the study of Mohanty in *Ailia coila, Anabas testudineus* and *Gudusia chapra* done by ICP-AES and AAS but was detected in *Amblypharyngodon mola* (0.2±0.0 mg/100 g), *Puntius sophore* (0.1±0.0 mg/100 g) done by ICP-MS.[11] However, in the present finding, it was comparatively higher than that of the reported range of 0.226±0.01 mg/100 g to 0.47±0.007 mg/100 g by Shantosh and Sarojnalini.[10] The concentration of zinc was also comparatively higher than the study reported by Hei A, 2013[29] and lower than the fish species *Gudusia chapra* (12.3±2.3mg/100 g) and *Ailia coila* (10.2±2.1 mg/100 g) (Mohanty *et al*.).[11]

Manganese is essential in bone mineralization, protein and energy metabolism, glycosaminoglycan synthesis, metabolic regulation and cellular defense against free radicals.[30] The concentration of Mn content in the present study shows slightly higher values than large fish such as *Catla catla, Cirrhinus mrigala* and *Labeo rohita* (NUTRIFISHIN ICAR).[31] Also, the concentration found in the study is higher as compared to those of *Devario yuensis, Glossogobius giuris, Hypsibarbus myitkyinae, Puntius chola* and *Tariqilabeo burmanicus* (Mayanglambam S and Chungkham S, 2018).[12] Higher concentration may be due to analysis of fishes as whole body. Mn is found in the liver, muscle, skin, gonadal tissue and bone (John E.H.,2013).[32] Similarly, the concentration of *Ailia coila* (1.3±0.9 mg/100 g), *Amblypharyngodon mola* (1.1±0.4 mg/100 g), *Anabas testudineus* (0.8±0.4 mg/100 g), *Gudusia chapra* (4.61±1.3 mg/100 g), *Puntius sophore* (1.1±0.7 mg/100 g) by Mohanty *et al.*,[11] has been analysed by ICP-AES, ICP/MS and AAS. The values are almost in agreement with the present study except for the *Gudusia chapra*.

Ni and Se are present in concentrations below 0.01 ppm Selenium is widely recognized as a vital micronutrient for salmonids and a toxicant in food and water.[1] The element is transformed into more bioavailable organic forms in all biological systems, primarily as two selenoamino acids, selenocysteine (SeC) and selenomethionine (SeMet). All selenium proteins have at least one SeC and are responsible for a variety of biological processes.[33] SeC is a necessary component of other selenoproteins and is found in vertebrates at the active sites of thioredoxin reductases, iodothyronine deiodinases, glutathione peroxidases and selenophosphate synthetases.[33] Se is also not detected in the study by Mohanty, in *Ailia coila, Amblypharyngodon mola, Gudusia chapra, Puntius sophore,* however, detected in *Anabas testudineus* (0.3± 0.2 mg/100 g) done through AAS. Cobalt is present in trace amounts in pre-monsoon season (0.0113±0.0023 mg/100 g) but not detected in monsoon and post-monsoon season in males. In females, it is found in monsoon (0.1393±0.011 mg/100 g) and post-monsoon season (0.2790±0.0101 mg/100 g) but could not be detected in pre-monsoon season. Co is a component of vitamin B_{12} (cobalamin). The metabollically active forms of this vitamin are methylcobalamin and 5-deoxyadenosylcobalamin. Two other forms, hydroxocobalamin and cyanocobalamin, are converted to these active forms-methylcobalamin and 5-deoxyadenosylcobalamin. Fishes require vitamin B_{12} as they cannot synthesize this vitamin from dietary Co sufficiently by microbiota in their digestive tract.[1] Chromium is a transition metal that differs significantly in its bioavailability and toxicity by existing in food and the environment in two forms- Cr^{3+} (trivalent) and Cr^{6+} (hexavalent). Cr is present in all the samples in trace amounts, ranging from 0.081 mg/100 g to 0.179 mg/100 g in males and from 0.116 mg/100 g to 0.2308 mg/100 g in females. The maximum concentration is found in the monsoon season in both males and females. According to the definition of an "essential trace element", no abnormalities are produced by its absence or deficiency from the diet. Some specific studies on the effect of Cr in fish have been related to its role in metabolism, growth and toxicity.[1]

CONCLUSION

ICP-AES analyzed 13 elements in this work. Overall, this study confirmed that *Lepidocephalichthys berdmorei* is a good source of nutrients besides the seasonal as well as gender variations. The aquatic environment is directly influenced by the minerals discharged from uneaten feeds and undigested materials excreted in feces and urine from aquaculture operations and hatcheries,[34] when excreted in soluble and particulate forms, affect water quality by settling to the bottom of ponds or tanks or by accumulating at the end of raceways. The breakdown of organically bound minerals in feces and the amount of soluble inorganic compounds from urine in water is significantly affected by the chemical composition of feedstuffs and inorganic or organic mineral supplements. Microorganisms and several environmental factors such as temperature, pH water current, dissolved oxygen levels and salinity also affect minerals released from feces and urine in natural waters. *Lepidocephalichthys berdmorei* is being consumed in the region because of its delicacy and health benefits. The nutritional values of this fish are very high which will also be supported in this study. In recent years, the fish population has dwindled in the wild and their price in the market is very high. Although it is listed under the Least Concern category of the IUCN Redlist, there is a need for further assessment. Studying the life traits as well as understanding the nutritional value of the fish can help in judicious management and conservation.

As the aquatic environment is complex and governed by numerous variables, a laboratory case study mimicking freshwater with varying parameters (pH, temperature, oxidation state, etc.) can help study the respective effects on selected elements and would help further understand the effect of each variable on metals accumulation in various fishes in further studies. Further studies on the effect of seasonal and temporal variations on each element also need to be conducted.

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ETHICS APPROVAL

The Institutional Animal Ethics Committee (IAEC) of Manipur University approved the work as this is part of the thesis work of the first author.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUMMARY

The mineral content in male and female *Lepidocephalichthys berdmorei*, a hill stream loach from Manipur, India, was analyzed during three seasons by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) and variations of 8 trace elements (Fe, Zn, Mn, Cu, Co, Ni, Cr, Se) and 5 macro-elements (Ca, P, K, Na, Mg) as the parameters. In males, the highest value was observed in Ca (4895.95±5.01 mg/100 g) and lowest in Co (0.0113±0.0023 mg/100 g). The values of trace elements- Fe (8.03±0.32 mg/100 g) and Zn (4.62±0.91 mg/100 g) were also significantly higher. In females, Ca (3712.52±9.08 mg/100 g) had the highest value among the macro elements, the lowest value was found in Co at 0.013±0.00 mg/100 g, values of trace elements Fe (11.72±0.41 mg/100 g), Zn (5.42±0.33 mg/100 g) were also found significantly high. Seasonal variations of Ca, P and K in males were observed highest in post-monsoon and Ca and P varied in females. In both sexes, Ni and Se were below 0.01 ppm. Our study highlights the importance of minerals in the growth and health of fish and the need for sustainable conservation strategies, providing valuable insights to nutritionists, researchers and conservationists.

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