Serum levels of macro minerals (trace elements) in some local fresh water fishes during breeding period

RS Kulkarni, Vijayakumar K and Pruthviraj CB

DOI: https://doi.org/10.22271/fish.2021.v9.i5b.2576

Abstract

The role of macro minerals (trace mineral elements) in biological systems has been described in several animals. However, the knowledge in fish is mainly limited to iron, copper, manganese, zinc and selenium as components of body fluids, cofactors in enzymatic reactions, structural units of non-enzymatic macromolecules, etc. Investigations in fish are comparatively complicated as both dietary intake and water borne mineral uptake have to be considered in deterring the mineral budgets. The importance of trace minerals as essential ingredients in diets, although in small quantities, is also evident in fish. Serum levels of some important macro minerals such as sodium (Na\(^+\)), potassium (K\(^+\)), calcium (Ca\(^+\)), phosphorous and magnesium (Mg) have been studied in four freshwater fishes collected from a local market as these fishes are edible fishes and are extensively purchased by the local public in the Kalaburagi city. These minerals play significant role in accumulation of nutrients, osmoregulation, homeostasis and other physiological activities of the fish particularly during breeding period. The blood serum level of these macro minerals determined in four local fresh water fishes are, <i>Labo rohitu</i>, <i>Catla catla</i>, <i>Cirrhina mrigala</i> and <i>Pangusius becucti</i>. The results of macro mineral concentration level were compared among them and it is indicated that there is difference. The serum sodium level in all the four types of fishes is at higher level in comparison to other minerals. The variation and the order of concentration of minerals in all four types of fishes found to be as: sodium> potassium> phosphorous> calcium> magnesium. The aquatic body in which these fishes are harbored has required mineral concentration needed for various physiological activities in the process of nutritional absorption, osmoregulation and homeostatic mechanisms. Hence, the levels of blood serum macro minerals found in these fishes are though lesser during breeding period in the present study are considered to be in normal levels as reported in other fishes.

Keywords: Serum, macro, minerals, trace elements, <i>Pangusius becucti</i>

Introduction

Blood chemistry and haematological measurements can provide valuable tools for monitoring the health condition of both wild and cultured fish. The serum macro minerals concentration play significant role in various physiological activities and for fish survival. As macro minerals (blood electrolytes) Na\(^+\), K\(^+\), P\(^-\), Mg and Ca\(^+\) levels indicate the operation of a variety of homeostatic mechanisms in the body (Clarke, 1998) \(^2\). Sodium (Na\(^+\)), potassium (K\(^+\)) and Calcium (Ca) play an important role in osmoregulation and homeostasis. In vertebrates, the Na\(^+\) concentration in the extracellular fluid surpasses that in the cytosol, whereas K\(^+\) is higher in the intracellular fluid compared to the plasma. In fish, Na\(^+\) enters the gill cells from the blood; co-transported with K\(^+\) and Cl\(^-\) and driven by an electrochemical gradient favorable to Na\(^+\). Chlorine (Cl \(^-\)) exits the apical portion of the cell through a channel that is very similar to the defective structure that produces cystic fibrosis in animals. Na\(^+\) is transported back across the baso-lateral membrane into the blood by Na\(^+\)/ K\(^+\) activated ATPase. Furthermore, the Na\(^+\)/ K\(^+\) ratio is vital for the ion permeability barriers in the cell membrane. (Evans, 1993) \(^3\). One of the divalent ions, calcium (Ca\(^++\)), serves a number of functions in fish. It combines with phosphorus (P\(^-\)) for the deposition in the bone. It is possible that bone serves as a reservoir of calcium for plasma and tissues. Additionally, Ca\(^++\)appears to be important in the reproduction and mitochondrial functions. It is generally recognized that Ca\(^++\) has an important role in osmoregulation (Worst and Stickney, 1989) \(^4\).
The physical and chemical changes in aqueous environment often cause some physiological changes in fish, thus, the water quality of an aquatic body is very crucial because it determines the productivity and other parameters necessary for fish survival. Many countries have legislated against the use of chemical poisons in aquatic systems and instead have policies favoring the use of natural bio-degradable alternatives to remove unwanted fish species in aquatic systems. Environmental factors such as pH, turbidity, alkalinity, dissolved oxygen, temperature and conductivity influence the rate of reaction of pollutants entering the water or the lethal effects on the aquatic organisms (Fagbenro, 2002) [4]. Water temperature affects all aspects of metabolism and at high temperatures metabolic rate increases alkalinity, acidity and pH determine fish health and the well-being of fish (Ross and Ross, 2002) [11]. In the present study blood levels of sodium, potassium, calcium, phosphorous and magnesium levels have been determined in relation to a reproductive stage of some locally available fish species collected from fish market and are cultured in the aquatic bodies of Kalaburagi.

Material and Methods
The blood serum levels of different macro minerals are determined in four types of fresh water fishes such as *Labeo rohita*, *Catla catla*, *Cirrhina mrigala* and *Pangasius becouri*. The macro minerals are sodium, potassium, calcium, phosphorous and magnesium. The serum of these four fishes was collected from the local fish market site by using 2cm disposable syringe and a 21 gauge disposable hypodermic needle. The blood was taken under gentle aspiration until about 2 ml has been obtained and blood serum was prepared by centrifugation and then used for the determination of macro minerals in a laboratory by a trained technician. Sodium and Potassium are determined by colorimetric method using commercial kit available in the market, Sodium is precipitated as triple salt with magnesium and uranyl acetate. The excess of uranyl ions are reacted with ferrocyanide in an acidic medium to develop a brownish colour. The intensity of the colour produced is inversely proportional to the concentration of sodium in the sample. Potassium reacts with sodium tetra phenyl boron in a specially prepared buffer to form a colloidal suspension. The amount of turbidity produced is directly proportional to concentration of potassium in the sample. Serum calcium and magnesium was estimated by OCPC method provided by Gitelman *et al* (1967). Serum was diluted in 1.5 ml with deionised water in the 0.1ml diluted serum, 2ml of CPC reagent was added followed by 2ml of dimethyl amine solution. All tubes were mixed by continuous invasion and then tubes were left for 5 minutes at room temperature. The purple colour developed and was measured at 575 nm for calcium and magnesium concentration in the serum and expressed as mg/dl. Phosphorus is determined by Molybdate UV method. (Using commercial kit available in the market), When inorganic phosphorus reacts with ammonium Molybdate in an acidic medium to form a phosphomolybdate complex which is measured in UV range i.e., at 340 nm. The absorbance of the complex is directly proportional to the amount of phosphorus present in the sample.

Statistical treatment of the data
The obtained data was analyzed statistically by adopting varied statistical methods. Standard deviation and the student’s- t’ test was carried out to know the levels of significance using the standard formula. All the values of P below 5% level are designated as significant, and the values above 5% level are designated as non-significant (Mungikar, 2003) [7].

Results
The macro minerals are most important components which influence the distribution and retention of body water. Sodium (chief cat ion of extracellular fluid), and potassium (chief cat ion of intracellular fluid) are the most important osmotically effective macro minerals and act as electrolytes. The range of sodium in the four fishes is higher (70.65 to 94.0 with an overall mean of 85.33mmol/dl) during breeding period, whereas the sodium level was higher during non breeding period reported earlier in the fresh water fish, *Notopterus notopterus* (57.5 -101.32 mmol/L) with an overall mean of 91.27 mmol/L (Kulkarni, 2015) [6]. The potassium range is 16.6 -39.4 mmol/L with an overall mean of 26.0 mmol/L. Phosphorous is combined with calcium in bones. It is found in every cell of the body and some amount is combined with proteins, lipids, carbohydrate and other compounds in blood and muscle in the fishes. Phosphorous content is found moderate in the range of 02.05 – 10.00 mg/dl in the blood of fishes studied in the present investigation with an overall mean of 6.00mg/dl. The magnesium range found to be in the range of 2.84 – 10.00 with overall mean of 6.24mg/dl. The values are presented in the Table – 01 and fig.-01. The ionic compositions of water are important for all fishes. The concentration of divalent cat ions Ca**++* and Mg**++* play vital role in the ionic regulation of fresh water fishes because these ions modulate bronchial permeability as water is also an important source for Ca**++* and Mg**++* required for fish growth.

Discussion
The role of macro minerals (trace mineral elements) in biological systems has been described in several animals. However, the knowledge in fish is mainly limited to micro minerals such as iron, copper, manganese, zinc and selenium as components of body fluids and they act as cofactors in enzymatic reactions, structural units of non- enzymatic macromolecules, etc. Investigations in fish are comparatively complicated as both dietary intake and water borne mineral uptake have to be considered in determining the mineral budgets. The importance of trace minerals as essential ingredients in diets, although in small quantities, is also evident in fish.

The serum macro minerals which are also called trace elements such as sodium (Na*+*), potassium (K*+*), calcium (Ca**++*), magnesium (Mg**++*), phosphorus (P**) are commonly used to determine the physiological characteristics, toxicity and health status of fish (Percin et al., 2010) [9]. The monovalent ions namely sodium (Na*+*), potassium (K*+*) and chloride (Cl-) play an important role in osmoregulation and homeostasis. The biochemical concentration of these macro mineral trace elements in the serum of the four fresh water fishes reported in this communication are found to be in normal levels. In some of the reported fishes such as *Acipenser stellatus* (Shahasvani et al., 2010) [12] in which Na*, K*, Ca**, P* are at higher concentration, since this fish is a marine fish. In another fish from marine habitat, black scorpion *Scorpaena porcus* reported by Celik, (2004) [1]. The serum concentration of these macro minerals were also at higher level. In large number of fishes studied, the Na* levels

---

International Journal of Fisheries and Aquatic Studies

http://www.fisheriesjournal.com

~ 125 ~
were higher, the values in *Scophthalmus niloticus* (157.00 ± 0.3 – 186.00 ± 0.4 mmol/l), *Oreochromis niloticus* (161.8 ± 4.2 mmol/l), *Piaractus brachypomus* (150.4 mmol/l) *Acipenser naccarii* (140.6 ± 4.8) and *Salmo salar* (137 ± 1.1 – 196 ± 18.6mmol/l). The K levels were lower than the values in *Scophthalmus aquosus* 4.1 ± 0.18 – 5.48 ± 0.15 mmol/l), *Oreochromis niloticus* (4.83 ± 1.15 mmol/l) and *Pagas auratus* (6.0 mmol/l) but were higher than these of *Salmo salar* (1.3 ± 0.4 – 4.5 ± 0.1 mmol/l). Calcium concentration was lower than values reported in *Oreochromis niloticus* (17.43 ± 6.02 mg/dl) but were higher than *Pagas auratus* (3.1 mg/dl) *Piaractus brachypomus* (10.80 mg/dl) and *Acipenser naccarii* (2.3 ± 0.1) and Ca**⁺** values were similar to those reported in *Scophthalmus aquosus* 3.49 ± 0.11 – 4.43 ± 0.10 mg/dl and *Salmo salar* (3.3 ± 0.1 – 4.7 ± 1.4 mg/dl) and suggested that the levels of macro minerals (Na⁺, K⁺, Ca**⁺**, and P⁴) indicate the operation of a variety of homeostatic mechanisms in the body (Clarke, 1998) [2]. Potts and Rudy, (1972) [10] measured some of these serum parameters, in green sturgeon (*A. medirostris*), in which Na⁺ (114 mmol/l), K⁺ (1.5 mmol/l) and Ca**⁺** (2.6 mmol/l). Sodium levels were found higher in all the four fishes studied in the present study. The potassium (K⁺) found lover in comparison to calcium (Ca**⁺**) in all these fishes. However, variation in the concentration of calcium, phorphorous and magnesium among the fishes was noticed may be because of difference in the absorption capacity of the fish. Holmes and Donaldson, (1969) [5] measured some these parameters in *Acipenser oxyrinchus* (Na⁺: 151 mmol/l, K⁺: 2.7 mmol/l and Ca**⁺**: 1.9 mmol/l). In the present study, the Na⁺ levels were higher and K⁺ levels were lower. Natochin et al., (2000) monitored the biochemical parameters of blood serum in Russian sturgeon, *Acipenser gueldenstaedtii* from 1974 through 1993, the reported values for Na⁺ (115.40–165.00 mmol/l) was higher. According to the latter research, the Ca**⁺**/Na⁺ ratio can be used as an indicator of the condition of fish. The values obtained for various macro minerals in the present study for the Indian carp fresh water fishes were found to be normal values as these fishes are living in fresh waters and source of these macro minerals may be less in comparison to the fishes living in marine waters as mentioned above. However, the fishes were thriving well in the aquatic bodies of this region (Kalaburagi) indicating that they have healthy condition with favorable environmental conditions for reproductive activity.

Table 1: Showing blood serum macro mineral contents in some local freshwater fishes collected from aquatic bodies of Kalaburagi

| Name of the fish         | Sodium (Na⁺) in mmol/L | Potassium (K⁺) in mmol/L | Calcium (Ca**⁺**) in mg/dl | Phosphorous (P⁴) in mg/dl | Magnesium (Mg**⁺**) in mg/dl |
|--------------------------|------------------------|--------------------------|---------------------------|--------------------------|-----------------------------|
| *Labeo rohita*           | 91.3±0.61              | 28.6±0.30                | 8.7±0.22                  | 10.0±0.25                | 3.12±0.23                   |
| *Catla calla*            | 70.65±0.35             | 16.6±0.10                | 6.5±0.26                  | 2.25±0.17                | 10.0±0.18                   |
| *Cirrhana mirgala*       | 94.0±2.10              | 18.6±0.19                | 10.0±0.34                 | 2.05±0.02                | 10.0±0.23                   |
| *Pangusius becouri*      | 85.4±1.06              | 39.4±0.13                | 9.0±0.20                  | 10.0±0.16                | 2.84±0.07                   |

Each value is expressed as mean ± SD, N = 6. All values are significant P < 0.01

**Fig 1**: Showing serum macro minerals of the four fresh water fishes.

**Conclusions**

The serum levels of macro minerals (electrolytes) level has been studied in four species of Indian carp fishes such as *Labeo rohita, Catla calla, Cirrhana mirgala and Pangusius becouri* indicates that the values are normal in comparison to other fishes reported, as these are fresh water fishes and the source of macro minerals may be more or less in the aquatic bodies in which they are harboured. Thus indicating that all the four types of fishes are healthy and probably their osmregulation and homeostatic mechanism are functioning normally for successful reproduction and probably these fishes are not stressed. However, there is variation of values between the fish species and this could be the method of capture, age of fish, method of drawing blood and diet are all variables that should be considered in accepting study specimens and evaluating results.

**Acknowledgement**

The author R.S. Kulkarni is grateful to the Indian Science Congress Association, Kolkata for Asutosh Mookherjee Fellowship and Gulbarga University, Kalaburagi for the facilities to carry out this work.
References
1. Celik ES. Turkey J Bio. Sci 2004;4(6):716-719.
2. Clarke F. A review of the scientific justifications for maintaining the cetaceans in captivity. A report for the Whale and Dolphin Conservation Society (WDCS). 1998.
3. Evans DH. The Physiology of Fishes. 2nd Edn., CRC press, Boca Raton 1993, 49-73.
4. Fagbenro OA. Tilapia: fish for thought. 32nd Inaugural Lecture, Federal University of Technology, Akure, Nigeria 2002, 77.
5. Holmes WN, Donaldson EM. The body compartments and the distribution of electrolytes. J Fish physiol., London 1969;1:1-9.
6. Kulkarni RS. Comparative studies on blood electrolytes of the fresh water fish, N. notopterus fro three aquatic bodies. Int. J.natural Sciences 2015;40:1-5.
7. Mungikar AM. Biostatistical analysis, Saraswati Publ. Aurangabad 2003.
8. Natochin, YuV, Lukyanenko VI, Shakmatova Yel, Lavrova YeA, Metallow GF Twenty years (1970–1990) of monitoring the physicochemical parameters of blood serum of Russian sturgeon, Asipencer gueldenstaedti. J Ichtiol Vopr. Ikhtiol 1995;(35):108-118.
9. Percin F, Sibel K, Kursat F, Sahin S. Serum electrolytes of wild and captive Bluefin Tuna (Thunnus thynnus L.) in Turkish Seas J. Anim. Vet. Adv 2010;9(16):2207-2213.
10. Potts WT, Rudy PP. Aspects of osmotic ionic regulation in the sturgeon. J Exp Biol 1972;56:703-775.
11. Ross B, Ross LG. Anaesthetic and Seductive for Aquatic Animals.2nd Edition, Blackwell Science Ltd 2002.
12. Shahsavani D, Mohri M, Gholipour H. Determination of normal values of some blood serum enzymes in Acipenser stellatus (Pallas) Fish Physiol Biochem 2010; 36:39-43.
13. Wurst WA, Stickney RR. Responses of red drum (Sciaenopso cellatius) to calcium and magnesium concentrations in fresh and salt water. Aquaculture 1989;76:21-35.