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Proximate Composition & Mineral Content In Muscle Tissue Of *Lutjanus Johnii* (Bloch, 1792) Captured From Coastal Waters Of Mumbai, West Coast Of India

Hitesh U. Shingadia
Svkm's Mithibai College of Arts, Chauhan Institute of Science And Amrutben Jivanlal College Of Commerce And Economics (Autonomous)
Vile Parle - West Mumbai 400 056
Email: Hiteshshingadia26@Gmail.Com

Abstract:
Background: Seafood has long been recognized as a valuable source of high-quality protein in the human diet with the consolidation of various biochemical compositions viz., moisture, proteins, carbohydrates, fats, minerals, vitamins & many other essential constituents required to maintain the life processes. *Lutjanus johnii* (Bloch, 1792) occurs in abundance in the coastal waters of the Indo-West Pacific along the West Coast of India. Apart from aquaculture, fish is also used as gamefish & noted for its nutritious flesh.
Objective: Bioaccumulation of minerals through pathways like food ingestion, particulate matter, gills and integument ionic exchange. The minerals perhaps get absorbed into the blood and transported to the organs for either storage or excretion. With this objective the present investigation on the proximate composition of the muscle tissue of *Lutjanus johnii* was assessed to know the its variations.
Results: The occurrence of biochemical composition and minerals in muscle tissue of *Lutjanus johnii* showed seasonal variation. Total protein content of fish varied from 11.52-18.55 G% while, the carbohydrate content ranged from 1.05-2.41 G%. Highest amount of lipid content was observed to be 4.75 G% that gradually declined during the winter season with lowest value amounting to 2.47 G% in the month of October'21. Moisture and ash content ranged from 70.28-77.37 G% and 1.59-3.32 G% respectively. The bioaccumulation of the minerals was observed to vary seasonally and correlated with feeding habits, habitat and the bio-concentration capacity of fish.
Discussion: The study revealed that shellfishes collected from Versova fish landing centre, Mumbai nutritious for human consumption and with potential for generating revenue through export. However, the occurrence of contaminants other than the minerals under study cannot rule out the risk and needs to be further investigated before affirming these seafood commodities safe for consumption.
Conclusion: The importance of shellfish cannot be ruled out as it forms an essential rich nourishment to mankind. It is essential to periodically monitor these aquatic resource be for their safety on consumption. The shellfishes are a major component of the marine ecosystem with tremendous export potential; thus assessment of the minerals is primarily vital.
Key Words: Proximate & mineral composition, *Lutjanus johnii*, Versova fish landing center.

Introduction:

Fishes belonging to Family Lutjanidae are generally carnivorous, piscivores or plantivores and are found worldwide in tropical and subtropical regions of all oceans (Ahmed and Naim, 2008). *Lutjanus johnii* (Bloch, 1792) abundantly occur throughout the Indian Coast and is considered as an esteemed food fish having great potential for export. Generally yellow with a bronze to silvery sheen, shading to silvery white on belly and underside of the head. A large black blotch mainly above the lateral line below the anterior dorsal-fin rays (Allen, 1985). A round black spot, larger than eye, on back, mainly above lateral line, below anterior soft dorsal rays is a characteristic feature of the species (Bal & Rao, 1984). Adults mostly inhabits brackish water in rocky areas while juveniles take shelter in coral reefs at depths of 20-50m. The sea food like fin fishes and shell fishes have high nutritional and the repented benefits in addition to important source of protein, essential minerals, vitamins and un saturated fatty acids (Munro, 1982). Eating fish at least twice per week in order to reach the daily intake of Omega-3 fatty acids. For maximum growth, greater food intake, higher feed utilization, higher nutrient retention efficiency and for stable body conformation and composition in fish and feeding frequency play a vital role (Booth, 2008).

The island of Mumbai comprises a coastal belt of around 100 km along the Arabian Sea that forms one the richest marine resources of one of the maritime states of our country, name Maharashtra (Shingadia, 2016). These estuarine and coastal areas are among the most polluted and also the threatened marine ecosystems in the world (Halpern et. al., 2008). Metals such as iron, copper, zinc and manganese are prerequisites for metabolic activities in organisms, whereas arsenic, cadmium, chromium, mercury, nickel and lead cause toxicity (Shingadia, 2016). In the aquatic environment, food and feeding habits of marine organisms are correlated with transformation and accumulation of minerals (Shingadia, 2016) . The accumulation and distribution of these metals in sediments of aquatic environment and thereby adversely affecting the marine life (Mohiuddin et al., 2010).

Mineral accumulation occurs through various pathways such as ingestion of food, suspended particulate matter, ionic exchange through gills and integument (Shingadia & Vaidya, 2018). Due to these pathways, minerals probably get absorbed into the blood and transported to various organs for either storage or excretion (Shingadia & Vaidya, 2018). The essentiality of macro minerals (calcium, phosphorus, magnesium, sodium, potassium and chloride) and certain trace elements (cobalt, copper, iodine, iron, manganese selenium and zinc) have been confirmed in fish (Suttle, 2010; Santosh & Sadasivam, 2021). Minerals are known to interact with other nutrients due to their lability and tendency to form chemical bonds (Santosh & Sadasivam, 2021). Direct positive interactions between elements in structural processes such as the requirement of copper (Cu) and iron (Fe) for haemoglobin formation, calcium (Ca), phosphorus (P) and magnesium (Mg) for formation of bone hydroxyapatite and an interaction of Mn with Zn for the proper conformational shape of RNA molecules in the liver have been widely recognized (Santosh & Sadasivam, 2021). Antagonistic relationships are considered to occur when trace elements with a similar electronic configuration and ionic radius compete for binding sites, such as zinc (Zn) and cadmium (Cd) in metallothionein, and Mg/manganese (Mn) substitutions at active sites of enzymes (Santosh & Sadasivam, 2021).

Materials & Methods:

Proximate Composition: The present investigation relates to the proximate composition & mineral content of *Lutjanus johnii* (Bloch, 1792) also known as 'golden snapper'. The fish specimens were collected on monthly basis for a period of one year from August 2021 to July 2022 from Versova fish landing center (Andheri) & local fish market in Mumbai along west coast of India. The fishes were brought to the research laboratory of Zoology department of SVKM's Mithibai College, cleaned & authenticated as per Day, (1878); Bal & Rao, (1984); FAO, (2010). Total protein content was estimated by Lowry's method (1951), carbohydrate by Anthrone reagent method (Hedge & Hofreiter 1962), lipid by Folch method (1957) & moisture by AOAC method (2000). The moisture content of the fish was estimated by drying a knowing weight (1g) of fish tissue in a hot air oven at 105^0C for 24hrs. The differences in weight before & after drying are the amount of moisture present and the results are expressed in percentage of wet weight of the tissue. Ash content was estimated by incinerating the fish tissue using muffle furnace at 550-600ºC by AOAC method (2000).

Mineral Analysis of Tissue Sample: For digestion of tissue and estimation of minerals, analytical grade reagents were used. Dilution of all solutions was done by using ultra-pure water. The concentration of minerals viz. Calcium, Iron, Zinc and Selenium in muscle tissue of fish species were estimated by using Flame Atomic Absorption Spectrometry i.e., Direct Air-Acetylene Flame method (Paus, 1971; Willis, 1962). Muscle tissues from these fish samples were by using a mixture of concentrated HCI (Hydrochloric acid) and HNO_3 (Nitric acid) and heated over a sand bath to dryness (Shingadia & Vaidya, 2018). $HClO_4$ (Perchloric acid) was added and boiled until dense white fumes were released, cooled, and filtered using Whatman 42 filter paper and the final volume was raised adequately with deionized water (Shingadia & Vaidya, 2018). All the plastic wares and glass wares were cleaned by soaking in 2M Nitric acid for 48 hrs and first rinsed with distilled water and then with deionized water prior to use (Shingadia & Vaidya, 2018). Stock standard solutions of the said metals (1000 µg/mL Titrisol, Merck in 2% v/v Nitric acid) were used for the preparation of calibration standards (Shingadia & Vaidya, 2018).

Statistical analysis of data: The Statistical package program (IBM SPSS 20th Edition) was used for statistical analysis (Shingadia & Vaidya, 2018). Inter-heavy metal correlations in the fish muscle were investigated. The p-values of less than 0.05 and 0.01 were considered to indicate statistical significance (Shingadia & Vaidya, 2018).

Results & Discussion:

The major components of food are moisture, proteins, carbohydrates, fats, minerals & vitamins. Fish proteins can be broadly divided into three groups according to their solubility viz. sarcoplasmic proteins, myofibrillar proteins & connective tissue protein. Proteins provide the necessary materials for the repair & building of muscles & tissues. Amount of protein in fish tissue can be correlated to the phases of maturity and spawning. Carbohydrates & fats provide energy while minerals & vitamins keep the fish healthy & bones strong. The proportion in which different constituents of the body occur in the organism is called its 'Proximate Composition', the study of which helps to estimate the nutritional quality of the fish. Table 1; Fig. 2 depicts the biochemical composition of the fish varies, depending on several factors such as species, age, maturity, method of catch, fishing grounds, geographical regions, season of the year, anthropogenic activities in the environment, etc. Even within a single species in different portions of the body in the same fish, biochemical composition may vary significantly (Govindan, 1985; FAO, 1995; Emilia & Santos, 1996). These biochemical constituents along with other aspects of fishery science such as feeding & breeding biology of fish, habit & habitat study will provide a better insight into the sustainable management of these marine aquatic resources apart from health consequences of the consumer.

Proximate Composition of *Lutjanus johnii*:

The variation of moisture, protein, lipid, carbohydrate and ash contents observed are reported in Table:1; Fig. 2. The chemical composition of the different fish species showed variation depending on seasonal variation, migratory behaviour, sexual maturation, feeding cycles, etc. These factors are observed in wild, free-living fishes in the open Sea and inland waters (Shamsan, S. 2008). Fish of various species do not provide the same nutrients to the consumers with health benefits and the nutritive value of a fish varies with season. The protein content of the cell is considered as an important tool for evaluation of the physiological standards. Total lipids content is said to be a good indication of nutritional values of fish tissue.

Protein: Total protein content in *Lutjanus johnii* varied from 11.52-18.55 G% during the study period of twelve months from August 2021 to July 2022. The lowest value of protein content was reported in the month of July 2022 (11.52 G%) that showed gradual increase reaching maximum to 18.55 G% in the month of October 2021. Since monsoon coincides with the breeding season, the pre-monsoon elevation in the muscle protein content could be allocated to the gonad maturation in anticipation of increased energy requirement during the latter period. Similarly increase during winter supplements for growth. Takama et. al. (1999) reported that protein content was more in fishes during early summer and winter months corresponding to their maturity stages as observed in present investigation. Protein is essential for the sustenance of life and accordingly exists in the largest quantity of all nutrients as a component of the human body. The protein value in *P. vigil* was 15.75 to 20.16 %. The protein content of *P. pelagicus* and *P. sanguinolentus* was 0.47 to 15.91 % and 12.81 to 13.6 % respectively (Ravichandran et. al, 2011).

Carbohydrate: The carbohydrate content in *Lutjanus johnii* showed similar trend in values as that of proteins. The minimum carbohydrate content of 1.05 G% was reported during pre-breeding period in the month of April 2022 that could be due to increased energy demand for gonad maturation resulting in diversion of energy provided by carbohydrates. The maximum carbohydrate content was reported in August 2021 (2.41 G%) that represented the breeding phase of fish in the life cycle. In fish accumulation of glycogen and glucose have been reported during maturation that declines after spawning. A rise in carbohydrate level during post-spawning period indicates the mobilization of glycogen in liver & muscle of the fish for growth & maturation as observed in the present study. Carbohydrates are a group of organic compounds including sugars, starches and fibre, which is a major source of energy for animals. Carbohydrates in fishery products contain no dietary fibre but only glucides, the majority of which consist of glycogen. They also contain traces of glucose, fructose, sucrose and other mono and disaccharides (Pilla and Konathala, 2014). The variation in the carbohydrate content in shell fishes may be influenced by the climatic conditions.

Lipid: Lipids are energy reserves of the organisms. The highest lipid content in the muscle tissue of *Lutjanus johnii* was observed in the month of August 202l (4.75 G%) that gradually declined post breeding season with lowest value amounting to 2.47 G% in the month of October 2021. Decline in the values of lipids signify diversion of energy for the maturation & liberation of gametes. Gonads are rich in lipid content as stored energy metabolite. In the body of fish mobilization of lipids from various organs to gonads occurs during gonadal maturation. An increasing trend in total lipid content was observed post-spawning that could be attributed to the resorption of mature gonads thereby mobilizing the lipids to storage tissues in the body. Teraiya et. al (2013) observed that metabolites like Lipid in Ovary increase during the active process of gametogenesis in both the fish species. While the Glycogen level decreased in *Sillago sihama*, whereas in *Otolithus ruber*, these metabolites showed an increased trend.

Moisture: In the present study moisture content in muscle tissue of *Lutjanus johnii* ranged from 70.28-77.37 G%. Moisture contents in the Indian fishes generally vary between 70-80 %. In fatty fishes like *Lutjanus johnii*, moisture content decreases with increasing fat content & in oil sardine values as low as 56 % have been reported during seasons of maximum oil content. In general, an inverse relationship is observed between the moisture & fat contents of fishes. Similar observations are reported in the present investigation. The lower values reported during the summer months could be attributed to dehydration of muscle proteins. Madhu, et. al., (2013) reported that low water content is usually associated with relatively high fat content and vice-versa and noticed high value of protein and low value of lipid in marine fishes that corroborates with present findings.

Ash: Ash gives a measure of the mineral contents of the fish muscle. Ash content in the muscle tissue of fish ranged from 1.59-3.32 G% during the study period of twelve months. In human nutrition minerals are important, they are essential for body maintenance and some are a part of enzymes (Liu, 2002). The present result is in partially agreement with the result of (Chandrasekhar and Deosthal, 1993). Depending on the environment feeding habit and migration mineral content that contribute to the total ash content of the fishes may be change from place to place and region to season (Andres, 2000).

Mineral Analysis in Muscle Tissue of *Lutianus johnii:*

The mineral content in the muscle tissue of *Lutianus johnii* is depicted in Table 2; Fig. 3. In aquatic environment minerals in dissolved form are easily taken up by aquatic organisms where they strongly bind with sulfhydryl groups of proteins and accumulate in their tissues resulting in chronic illness and cause potential damage to the population (Shingadia & Vaidya, 2018). They also cause serious impairment in metabolic, physiological and structural systems when present in high concentration in the milieu (Shingadia & Vaidya, 2018). The bioaccumulation potential of these minerals was observed to be species specific and may be correlated to their feeding habits, habitat and the bio-concentration capacity of each species as reported by Fariba et al. (2009). The essential elements, such as boron, iron, zinc, copper and manganese are in adequate concentrations, presumably due to their function as co-factors for the activation of a variety of enzymes and regulated to maintain certain homeostatic mechanism in the fishes (Shingadia & Vaidya, 2018). The bioavailability of trace elements is a key factor in determination of concentration of metals in tissues of aquatic biota (Shingadia, 2016). Sea food forms the chief link for the probable relocation of these minerals into the human beings

(Shingadia, 2016). Information on the level of pollution by these minerals in coastal environment is essential as they are the source for deleterious environmental health hazards (Shingadia, 2016).

Calcium (Ca): Calcium content in the muscle tissue of *Lutjanus johnii* ranged from 18.03-21.84 mg/100g. Higher calcium content observed during monsoon season coincides with the breading season of the fish. Commonly, a large portion of the Ca requirement of most fish is encountered by its absorption through gills (Santosh & Sadasivam, 2021). In the diet of fishes such as carp, red sea bream, striped bass, tilapia, catfish and chum salmon concentration of calcium is observed (0.34 % or less) (Santosh & Sadasivam, 2021). Development and maintenance of the skeletal system is a major role of Calcium and Phosphorous and also many other physiological functions including the maintenance of acid-base equilibrium is performed by Calcium and Phosphorus (Santosh & Sadasivam, 2021). Zinc is considered essential for normal eye development in juvenile fish. In skeletal tissue, Ca and P are deposited as tricalcium phosphate $Ca_3(PO_4)_2$, which then undergoes further crystalline changes to form hydroxyapatite, $Ca_{10}(PO4)_6(OH)_2$, which is deposited in the organic matrix during mineralization (Santosh & Sadasivam, 2021). Fish absorb Ca and P from the surrounding aquatic environment via gills, gastrointestinal tract and integument; however, the gills represent the major site of Ca uptake (Evans & Claiborne, 2009). The Ca requirement of fish are affected by dietary factors (e.g., bioavailability, P level), uptake from water and species differences (Santosh & Sadasivam, 2021).

Iron (Fe): Iron content in the muscle tissue of *Lutjanus johnii* ranged from 0.31-0.55 mg/100g. Iron was found abstemiously stable in amount in fish studied during period of twelve months with marginal variation (Table 2; Fig.3). Accumulation of high quantities of iron in human beings result in haemochromatosis, characterized by impaired regulatory mechanisms and tissue damage (Santosh & Sadasivam, 2021). (Shingadia 2016) observed moderately abundant Iron content in all the species of fin fishes collected from Coastal waters of Mumbai when compared with marine waters of Turkey (Dural and Bickici, 2010) and Gulf of Aquaba, Red sea (Ahmed and Naim, 2008), but lower than fishes from Caspian Sea (Fariba et. al., 2009).

Zinc (Zn): Zinc content in the muscle tissue of *Lutjanus johnii* showed marginal variation throughout the study period that ranged from 0.57-0.83 mg/100g. Zn is essentially non-toxic trace metal that is vital for enzymatic activity as cofactor and nucleic acid synthesis (Shingadia, 2016). An excessive uptake and accumulation of Zn in the gills is also regulated through in Zn uptake mechanisms (Santosh & Sadasivam, 2021). Chelation of Zn with amino acids, such as histidine or cysteine which have a high affinity for Zn, may enhance absorption and distribution in the fish tissues (Santosh & Sadasivam, 2021). Shingadia (2016) reported variable concentration of Zinc in teleosts, *S. longiceps* showed highest concentration of Zn (2.2038 ppm), followed by *P. niger* (2.1669 ppm). The lowest concentration was observed in *E. tetradactylum* (0.7713 ppm). However, fishes from West Bengal Coast accumulate high level of zinc than fishes from Turkey (Dural and Bickici, 2010), Red sea (Ahmed and Naim, 2008), SE Coast of India (Raja et. al., 2009) and West Coast of India (Shingadia, 2016).

Selenium (Se): Selenium content in the muscle tissue of *Lutjanus johnii* ranged from 72.08-76.14 mg/100g. Muscle has moderate levels of Se content but accounts for the largest pool of Se in body (Santosh & Sadasivam, 2021). Selenium as an essential micronutrient for fishes that is widely recognized. In all biological systems, oxidative Se forms are converted into more bioavailable organic forms, mainly as seleno-amino acids seleno-cysteine (SeC) and seleno-methionine (SeMet). A comprehensive study of the identification and comparative analysis of vertebrate selenoproteomes has shown more than 45 selenoproteins in bony fishes as well as 38 selenoproteins in Zebrafish. The minimum Se requirement of fish varies with the form of Se (inorganic or organic) ingested (Santosh & Sadasivam, 2021). In coho salmon, based on growth, whole body and liver Se contents, the dietary Se requirement was found to be in the range of 0.39-0.43 mg kg^{-1} (Pacitti et. al. 2015). Elevated Se levels for rainbow trout, chinook salmon, fathead minnow, striped bass, bluegill and razorback sucker ranged from 2.4-70 mg Se kg^{-1} in feed and 47-472 µg L^{-1} of water (Sato et. al., 1983). Oxidative stress has been proposed as a main cause of excess dietary Se intake or exposures in fish (Berntssen et. al., 2017). Zee et. al. (2016) reported that in Atlantic salmon fed high levels of selenite and Se-Meth yeast, oxidative stress was a main driver for Se toxicity; however, white sturgeon and brown trout fed high levels of organic Se did not show oxidative stress (Santosh & Sadasivam, 2021).

Conclusion:

The proximate composition of *Lutjanus johnii* (Bloch, 1792) was studied for Moisture content, Protein, Lipid, Carbohydrates and Ash in the muscles tissues from Mumbai Coast. The data collected for one-year on proximate composition of the fish is not adequate as the environmental catastrophe augmented by anthropogenic activities might impact the growth & survival of the fish stock. The studies on the presence of minerals in fish will contribute availability of first-hand data on their levels in fish species having marketable significance. However, to draw most appropriate deductions, further experimentations should be carried so as to tap the source of these minerals and its impact ecosystem. Consequently, continuous & long term observations would give better understanding to draw favourable conclusions. Insight into the biochemical constituents of the fish tissue reveals the health condition of the fish under study as also provides momentous data from nutrition perspective of the local populace of the coastal India consuming the same as rich protein source.

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41. **Fig. 1: *Lutjanus johnii* (Bloch, 1792)**

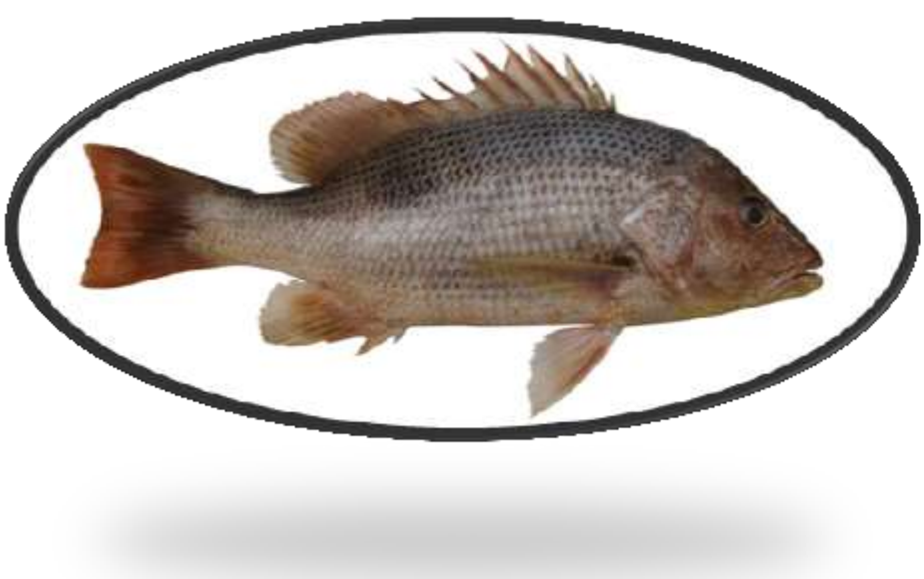

Table 1 Seasonal Variation in Proximate Composition of Muscle Tissue of *Lutjanus johnii* (Bloch, 1792)

Month	Protein (G%)	Carbohydrate (G%)	Lipid (G%)	Moisture (G%)	Ash (G%)
Aug'21	12.76 ± 0.431	2.41 ± 0.132	4.75 ± 0.715	73.08 ± 0.884	2.06 ± 0.932
Sept'21	16.53 ± 0.310	2.32 ± 0.742	4.42 ± 0.178	73.17 ± 1.962	2.85 ± 0.987
Oct'21	18.55 ± 0.582	2.04 ± 0.079	2.47 ± 0.234	75.97 ± 1.891	1.59 ± 0.106
Nov'21	17.84 ± 0.195	1.56 ± 0.148	2.91 ± 0.043	76.21 ± 1.977	1.86 ± 0.182
Dec'21	17.05 ± 0.536	1.25 ± 0.075	2.83 ± 0.274	77.37 ± 1.879	2.75 ± 0.171
Jan'22	16.72 ± 0.372	1.72 ± 0.193	2.97 ± 0.193	75.18 ± 1.532	2.83 ± 0.289
Feb'22	16.19 ± 0.982	2.34 ± 0.194	3.05 ± 0.228	74.92 ± 1.985	3.32 ± 0.401
Mar'22	15.94 ± 0.719	1.89 ± 0.179	3.82 ± 0.186	.66 ± 1.796	3.11 ± 0.302
Apr'22	15.96 ± 0.981	1.05 ± 0.561	3.46 ± 0.215	70.28 ± 1.859	2.74 ± 0.306
May'22	15.25 ± 0.892	2.14 ± 0.237	3.15 ± 0.273	74.94 ± 1.916	2.33 ± 0.253
Jun'22	14.64 ± 0.699	1.83 ± 0.404	3.36 ± 0.296	73.05 ± 1.795	1.94 ± 0.318
Jul'22	11.52 ± 0.278	1.59 ± 0.279	4.05 ± 0.146	71.87 ± 1.977	2.63 ± 0.243

All values are mean ± SE. ($P < 0.05$, $P < 0.01$) n=6

Fig. 2: Seasonal Variation in Proximate Composition of Muscle Tissue of *Lutjanus johnii* (Bloch, 1792)

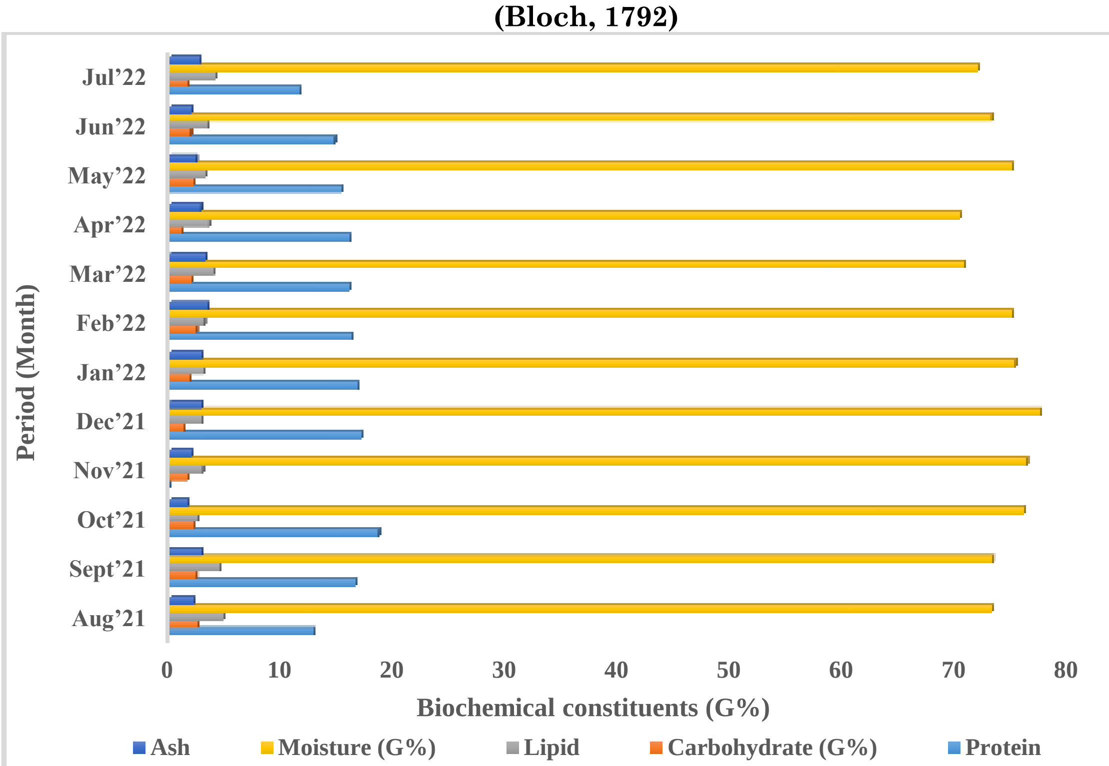

Table 2 Seasonal Variation in Mineral Content of Muscle Tissue of *Lutjanus johnii* (Bloch, 1792)

Month	Calcium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	Selenium (mg/100g)
Aug'21	18.03 ± 0.232	0.38 ± 0.312	0.65 ± 0.651	72.08 ± 0.943
Sept'21	21.32 ± 0.140	0.32 ± 0.524	0.62 ± 0.097	75.15 ± 1.782
Oct'21	20.36 ± 0.812	0.41 ± 0.701	0.57 ± 0.332	74.27 ± 1.495
Nov'21	20.74 ± 0.952	0.51 ± 0.245	0.59 ± 0.093	74.06 ± 1.036
Dec'21	21.84 ± 0.364	0.55 ± 0.016	0.81 ± 0.341	76.14 ± 0.989
Jan'22	19.79 ± 0.272	0.35 ± 0.392	0.77 ± 0.237	73.63 ± 1.639
Feb'22	19.42 ± 0.802	0.32 ± 0.156	0.72 ± 0.412	73.21 ± 0.915
Mar'22	18.68 ± 0.126	0.33 ± 0.276	0.64 ± 0.265	.44 ± 1.372
Apr'22	18.95 ± 0.802	0.31 ± 0.562	0.68 ± 0.356	74.02 ± 1.034
May'22	19.46 ± 0.933	0.37 ± 0.374	0.73 ± 0.371	74.95 ± 1.802
Jun'22	19.69 ± 0.276	0.45 ± 0.502	0.75 ± 0.942	75.05 ± 1.925
Jul'22	20.55 ± 0.371	0.51 ± 0.375	0.83 ± 0.047	76.04 ± 0.956

All values are mean ± SE. ($P < 0.05$, $P < 0.01$) n=6

Fig. 3 Seasonal Variation in Mineral Content of Muscle Tissue of *Lutjanus johnii* (Bloch, 1792)