Nutritional Profiling and Seasonal Comparison of the Bioactive Compounds in the Brackish Water Fishes of Goa: Green chromide and Grey mullet.

By

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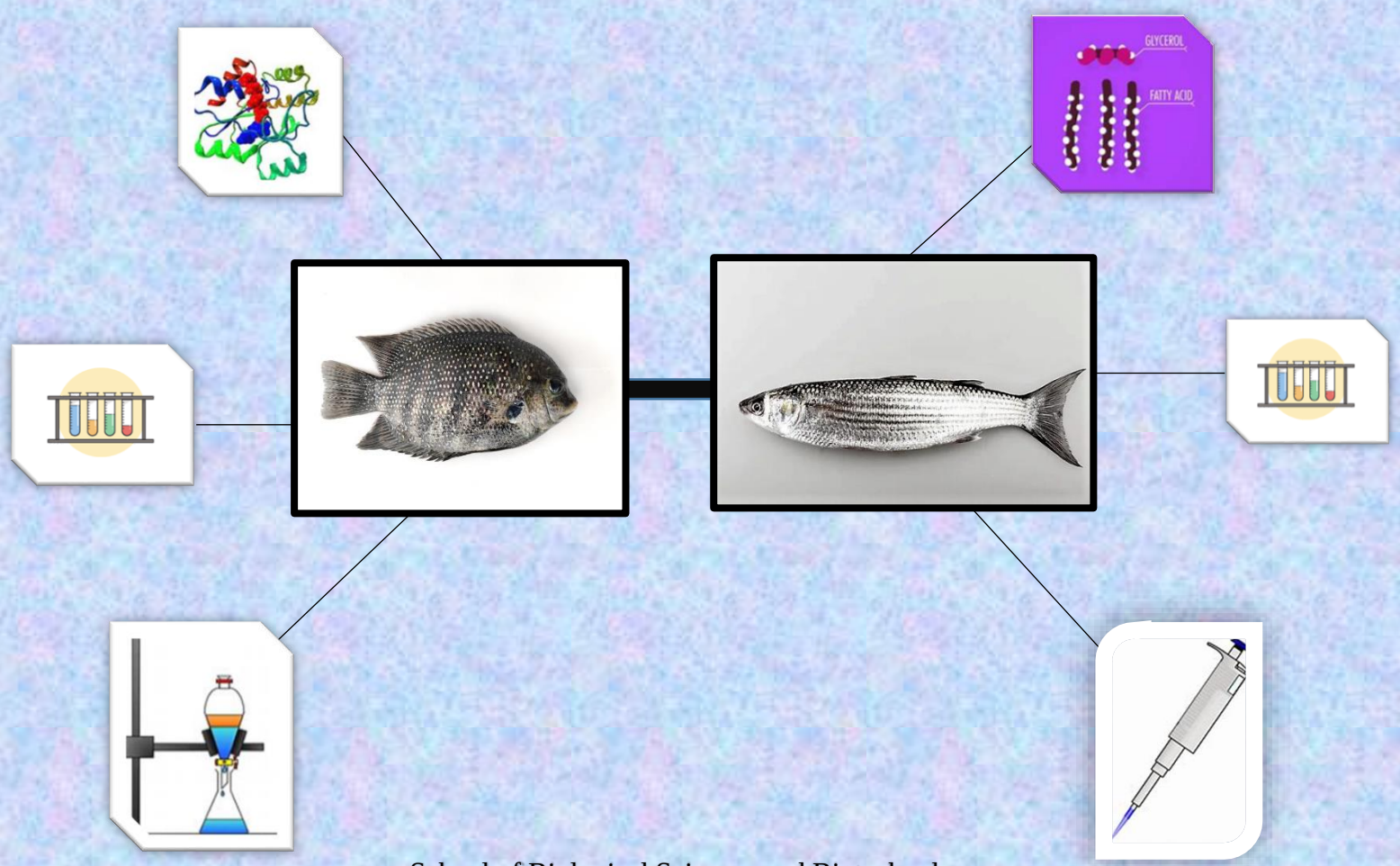

School of Biological Sciences and Biotechnology

Discipline Zoology

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COMPLETION CERTIFICATE

This is to certify that the dissertation "**Nutritional profiling and seasonal comparison of the bioactive compounds in the brackish water fishes of Goa: Green chromide and Grey mullet**" is a bonafide work carried out by **Ms. Vrunda Prashant Gayak** under my supervision/mentorship in partial fulfilment of the requirements for the award of the degree of **Masters of Science in Zoology** at the School of Biological Sciences and Biotechnology, Goa University.

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "**Nutritional profiling and seasonal comparison of the bioactive compounds in the brackish water fishes of Goa: Green chromide and Grey mullet**" is based on the results of investigations carried out by me in the Zoology Discipline at the, Goa University under the supervision/mentorship of **Ms. Gandhita Kundaikar** and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation. I hereby authorize the University authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

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INTRODUCTION

Introduction

Food is something that provides us with sufficient energy in order to carry out our daily activities and maintain our health. Proteins, carbohydrates, fats, fibre, vitamins, minerals and water are the essential nutrients required by the human beings. The process by which the organism assimilates food and uses it for growth and maintenance of cellular processes is called as nutrition. If all these nutrients are not consumed in sufficient amounts, then it can lead to reduction in the immunity, increased vulnerability to diseases, lessened physical and mental development and a reduced amount of productivity. Human nutrition is now becoming recognised as an important issue worldwide as many people are suffering from malnutrition and other diseases related to deficiency of nutrients. The main goal of nutritional science is to promote the eating of healthy food having abundant nutrients to prevent the common diseases due to nutritional deficiency like kwashiorkor and pellagra (Truswell, 2017).

The substances which give nourishment to the body and promote growth are referred to as nutrients. A very valuable and common source of nutrients to the humans are fishes. Early before the beginning of the civilization, fish has been an important component in the diet of the humans. Fishes are consumed by humans in a variety of ways in the form of different dishes. Due to its better availability and affordability, it is very useful for the people from the health point of view as it provides proteins, lipids, ash, minerals and vitamins. (Sujita Balami *et al*., 2019)

Essential nutrients are present in fish especially the proteins have high

biological value and thus it is often referred to as a rich food for poor people. It is better as compared to other animal proteins due to the presence of both macro as well as micronutrients. (Sujita Balami *et al*., 2019). The macronutrients which are present are proteins, carbohydrates, fats, fibre and water which are required in large amounts and the micronutrients present are vitamins and minerals, which are required in a smaller quantity. The working of these nutrients is in co-ordination with each other. Macronutrients provide energy to carry out the physiological and metabolic processes and provide energy whereas the micronutrients help in carrying out the metabolic processes of the body (Truswell, 2017). The proteins and fats present in fish are of major nutritional value. (Sujita Balami *et al*., 2019)

Macronutrients

1. Proteins

Numerous body functions have been known to be regulated by the proteins. Each protein is made up of many smaller units which are referred to as amino acids. Our body requires amino acids for preservation of proteins and maintenance. Amino acids are soluble in the digestive juices secreted by the small intestine and there they are absorbed into the bloodstream. They are unstable and hence cannot be stored in our body. So, once they are absorbed, they are either used as required or excreted out from the body. Therefore, protein should be consumed in sufficient amounts as our body does not store it like in the case of carbohydrates and fats. Protein plays a very important role in our body and is required for growth, development and repair of the tissues. Protein is a source of abundant energy reserves and it constitutes the hair, skin, nails and

the respiratory pigment haemoglobin in our body. Some hormones also contain proteins which are involved in maintenance of various bodily functions. All the enzymes in our body are proteins which help in catalysing various biochemical reactions and metabolic processes. Transportation of certain molecules across the cell membrane is also done by proteins. Another major function of the protein is the formation of antibodies which provides defence against various viral and bacterial diseases. (Truswell, 2017).

Various nutritional organisations give the values of the amount of protein required in the human diet. But the value can vary depending on the age, muscle mass, diet and current health status (Gunnars, 2017).

2. Carbohydrates

Carbohydrates provide a major source of energy for our body. They help in the breakdown of glucose in the body after which glucose moves from the bloodstream into the cells of the body with the aid of the hormone insulin. All the cells of the human body use glucose as an energy source to function. The human brain relies on a constant supply of 20-25% of glucose. The requirement of carbohydrates can vary according to the age, diet, weight and lifestyle of a person. If a person is thoughtful about the choice of carbohydrates, then this can provide a good balance of glucose and lessen the risk of related diseases. According to the food and Agriculture Organization (FAO), it is recommended that the people consume 275g of carbohydrate each day in a 2000 calorie diet (Bender, 2017).

3. Fats

Fats which are essential are very vital components of human nutrition. They help in reduction in the levels of cholesterol and phospholipids in the serum. Fats also help in forming the structure of the cell membrane and affect the function of the membrane bound enzymes and transport systems. They also modulate the cellular immune response. The important metabolites of essential fatty acids are prostaglandins, prostacyclins and thromboxane which are active compounds in the cardiovascular, lung, urinogenital and gastrointestinal system. Triglycerides, phospholipids and sterols also play a very important nutritional role. They are involved in the transportation and absorption of the fat-soluble vitamins that is vitamin A, D, E and K. The dietary reference intake for fats is 20%-35% of the total calories taken in from fat. It is recommended to include more of beneficial fats in our diet because they have a positive impact on our health. It is also been recommended to reduce the amount of harmful fats in our diet since they lead to a myriad of health issues (Gibney *et al.*, 2009)

4. Fibre

A polysaccharide that is incompletely absorbed by the body is referred to as dietary fibre. Depending on the solubility of fibre in water, they are categorised as soluble and insoluble. The consumed dietary fibre travels along the large intestine where the gut bacteria ferment it either partially or completely. During the course of fermentation various by-products like short chain fatty acids and gases are produced. The beneficial effects of dietary fibre on health are due to the combined action of fermentation process and the by-products. It is suggested

by several studies that fibre can help in the prevention of the risk of colon cancer. The main benefits provided by dietary fibre are proper bowel movement and improvement in the levels of blood glucose and blood cholesterol.

5. Water

Water acts as a medium for the occurrence of various biochemical reactions. It also acts as a means for the transport of nutrients and waste products. Water also plays a vital role in the maintenance of blood pressure, the volume of blood and the regulation of temperature. It has been suggested that consumption of 2 litres of water is necessary since 50-70% of our body weight is made up of water. It also helps in the prevention of dehydration. According to the National Academy of Medicine, 1 millilitre of water should be consumed for per calorie of food consumed. The daily intake for average adult male should be 3.7 litres and for females it is 2.7 litres.

Micronutrients

1. Vitamins

Vitamins act as coenzymes in several metabolic processes in the body. They are also involved in the synthesis of various compounds. According to their solubility vitamins are classified into fat-soluble and water-soluble vitamins. The fat-soluble vitamins are A, D, E, and K and the water-soluble vitamins are eight B complex and C. The vitamins which are water-soluble are not readily stored in our body and if they are in excess then they are readily excreted from

our body and because of this reason they are not toxic. Fat soluble vitamins are stored in the fatty tissues and if taken in excessive amounts can lead to toxic effects. Vitamins help in the development of embryo, reproduction, growth, immune response, building of connective tissues and bone metabolism.

The study analysed the levels of Vitamin C in fish.

Vitamin C is very important in the human diet because it acts as an antioxidant and is also an important cofactor for many enzymes. Humans lack the ability to synthesize this vitamin and hence it is obtained through the diet. It is a vital cofactor for biosynthesis of collagen, metabolism of carnitine and catecholamine and the absorption of iron in the diet. (Abdullah M. et al., 2022)

Other important vitamins in fish are:

Vitamin D is a very important nutrient. The receptors for vitamin D are located all over the body including the immune cells. It is also required for proper absorption of calcium and known to reduce risk of fractures because it helps in making the bones stronger and improves our balance and prevents us from falling. It has a role in keeping our lungs healthy because of its anti-inflammatory effect. Vitamin D also helps in the regulation of kidney function and plays a vital role in the treatment of kidney diseases (Amen D., 2018).

Vitamin E has antioxidant properties. It helps in the protection of our body from the damage caused by reactive oxygen species which cause harm to the cells, tissues, organs and even the DNA. It also makes our immune system more powerful against the bacteria and viruses. Another function of vitamin E is

formation of the RBCs, helping the body in the usage of Vitamin K and widening of blood vessels.

2. Minerals

The inorganic compounds which are found in our body as ions or as part of complex molecules are called as minerals. Around 17 minerals re required for the maintenance of normal metabolic processes. Minerals are divided into 2 categories that is major minerals and trace minerals. Major minerals are calcium, phosphorus, sulphur, sodium, chloride, magnesium and potassium. These are needed in amounts more than 100mg. Iron, zinc, copper, manganese, iodine, selenium, fluoride, molybdenum, chromium and cobalt are present in scarce amount and hence known as trace minerals. These are needed in less amounts. Calcium helps in maintaining the bone health and potassium keeps the muscles and nervous system strong. Sodium assists in the regulation of fluid-alkali balance. Zinc helps the immune system in providing the defence against infections (Mary L. *et al*., 2015).

Fish as a source of nutrition

Fish is a source of abundant amount of several nutrients. It contains good amount of lean protein therefore it is more valued over animal meat and often called as a rich food for poor people.

Fishes contain 15-20% protein of their body weight. All the essential amino acids are present in the fish especially the cysteine and methionine which are

absent in plant proteins and these help in the improvement of the nutritional quality of the mixed diet. Immunoglobins are found to occur in the proteins in fish whose role is to provide defence against the bacterial and viral infections. According to Mohanty (2015), the proteins in fish also help in prevention of the protein calorie malnutrition. Also, this protein source is more digestible as compared to animal protein due to the lower level of connective tissue present. (Sujita Balami *et al*., 2019)

The food type of the fishes will largely influence the nature and quality of the nutrients in the fish. The nutritional composition of the flesh of the fish is largely affected by the feeding habit of the individual species of fish. About 85-90% of protein in fish is digestible and the measurement of the approximate composition of the proteins is often necessary so as to ensure certain food regulatory requirements. (Sanatan Singh1*, 2016)

According to the species of fish, the diet and different environmental factors like salinity, temperature, geographical location and whether the fish is wild or farmed, the composition of the fatty acids vary. Fish lipids have the presence of polyunsaturated fatty acids like docosahexanoic acid and eicosapentanoic acid. Polyunsaturated fatty acids are very essential and prevent various diseases. They perform important functions like decreasing the rate of myocardial infarction and lowering the blood pressure and triglyceride concentration in blood. The lipids present in fish also help in the averting certain cardiovascular diseases. The fatty fishes contain omega-3 fatty acids which is important for the growth of children and also helps in prevention of the coronary artery disease.

Fishes have the presence of various minerals which are not present in other foods. Like milk and milk products, fishes also have a good amount of calcium in their bones and flesh. Fish also has abundant amounts of other minerals like selenium and iodine. Some small fishes which are consumed wholly contains large amounts of beneficial minerals like iodine, selenium, zinc, iron, calcium, phosphorus and potassium. In large doses, selenium is toxic, but it is required in small amounts as it acts as a cofactor for reducing the antioxidant enzymes like glutathione peroxidase. Iron is very important for the synthesis of haemoglobin which transports the oxygen all over our body.

Fish is also a really good source of essential nutrients but the amount present in each species of fish may vary. It has several vitamins like vitamin A, D and several B complex vitamins. Large amounts of vitamin A and D is stored in the liver of many species of fish. Vitamin D is found to occur naturally in fish oils and fish foods. (Sujita Balami *et al*., 2019)

Commonly found fishes of Goa were chosen for the protein estimation. Green chromide (*Etroplus suratensis*) and Flathead grey mullet (*Mugil cephalus*) were chosen for the study purpose. Green chromide is greenish brown fish with 6 dark vertical bars and Grey mullet has a silvery, elongated body. Both these fishes were collected from a brackish water sluice gate. Brackish water fishes can tolerate a highly saline environment. (Saleh M. A., 2009). Different kinds of nutrients are found in fish which have different protective functions in the human body. The study aims to provide information on the comparison of the bioactive compounds in Green chromide and Flat head Grey mullet and the value of these to human health. Such type of information can

make people aware about the health benefits of consumption of fish and its role in prevention of various diseases. (Sujita Balami *et al*., 2019)

Review of literature

Fish is a very valuable food source due to good balance of all the essential nutrients like proteins, lipids, minerals and vitamins. But people are still unaware about its essential benefits and so they should be made aware by conducting various studies on the nutrients present in fish and assessing their health benefits (Sujita Balami *et al*., 2019).

Protein profiling was carried out in 8 different species of snakehead fish in order to study the phylogenetic relationship. It was stated by Haniffa M. A. *et al*., (2017) that a total of 62 bands were observed by SDS-PAGE and the highest number that is 12 were observed in *Channa gachua*.

The protein and lipids content in different fish was analysed by Renata Pyz-Łukasik *et al*., (2020) and the data indicated that the protein and fat content in the muscles depend on different factors like diet, body weight, environment, catch season and farming system.

According to Mohanty (2015), the protein in fishes can also help to prevent diseases like kwashiorkor and marasmus which cause malnutrition in children.

The amount of protein present in the muscle tissue of *Mugil cephalus* was investigated by using Lowry's method, GC-MC analysis and SDS-PAGE in order evaluate the antimicrobial activity of the tissue. It was stated by B. Deivasigamani *et al*., (2017) that the protein profile of *Mugil cephalus* showed protein that ranged from 100 kilo Daltons to 10 kilo Daltons.

In another study the lipid and protein content was analysed in 3 freshwater teleosts. The protein content was estimated by using Lowry's method and it was observed by Sanathan *et al*., (2016) that the nutrient content in the tissues of the fish depends on season, age, sex, reproductive cycle and the breeding season.

An analysis of protein and fatty acid profile of 9 marine fishes was conducted which were collected from a fish market in Java, Indonesia. It was stated by Sri Priatni *et al*., 2018 that the total protein content of fishes ranged from 61.07% to 86.56%.

In another study the nutrient composition, the protein and fatty acid profiles of the fish by products like heads, gills, intestines, bones, trimmings and skin were measured. It was concluded by Aikaterini Kandyliari *et al*., (2020) that the protein content was 44.27±13.35%.

The nutrient profile of the commonly available fishes of Lakshadweep Island was analysed. The protein, carbohydrate, lipid, ash, vitamin, amino acid and fatty acid composition of the fishes was studied. The analysis revealed that the protein, carbohydrate, lipid and ash contents were reported to be high in *Thunnus albacares*, *Parupeneus bifasciatus*, and *Hyporhamphus dussumieri* respectively. Lysine, leucine and methionine were the major essential amino acids that were observed in the fishes. The fishes also contained saturated, monounsaturated and polyunsaturated fatty acids. This data given recorded by the authors (Kottila Veettil Dhaneesh *et al*., 2012) shows that the fishes which

were studied are full of nutrition and therefore were recommended for consumption.

The composition of the lipids and fatty acids was studied by (Misir *et al*., 2013) in the endemic pearl mullet found in the Lake Van of Eastern Anatolia in Turkey. It was found that the average lipid content of the fishes collected in September and November 2012 and January 2013 was 2.19g. The average content of SFA, MUFA and PUFAs determined in the collected fish samples were 21.88%, 44.37% and 29.19% respectively and the predominant ones were observed to be palmitic acid and oleic acid. The data revealed that the pearl mullet was also a good source of EPA and DHA and therefore were a really good source of nutrition for the human consumption.

A study was conducted by Chen T.C et al., in 2007 to see whether the vitamin D content in fish was enough to supply the dietary requirement. The vitamin D content in farmed and wild/caught salmon, blue fish, white fish, trout and tuna was calculated. In wild salmon the average vitamin D content was found to be 988±524 IU of vitamin D_3 which is the usual amount served for dinner. In the farmed salmon it was 240±180 IU of vitamin D_3 and in four different samples of blue fish the average vitamin D_3 content was found to be 280±68 IU. The white fishes like cod and gray sole were observed to contain 104±24 IU and 56±36 of vitamin D3 content. The vitamin D_3 content in farmed trout and tuna was found to be 388±212 IU of vitamin D_3 and 404±440 IU of vitamin D_3. After observing the values, it was concluded that these fishes are good sources of

vitamin D and therefore were recommended to include in the diet of the people having vitamin D deficiency.

Another study was conducted to determine the fatty acids contents in 20 species of marine fish and four shellfish species. The extraction of fat was done by following the method of Bligh and Dyer and the data suggested that most of the samples contained alpha linoleic acid, EPA and DHA. A high amount of omega-3 fatty acids was found to occur in Fringescale sardinella, Malabar red snapper, Black pomfret, Japanese threadfin bream, Giant Sea perch and Sixbar grouper. Due to the presence of omega-3 fatty acids, these fishes were highly recommended for lowering the high blood pressure (Abd. Aziz *et al*., 2013).

The fatty acid content present in five species of fish in the river Niger in the Edo state of Nigeria was analysed by Gas-Liquid Chromatography. The fish samples showed the presence of abundant amount of stearic acid, palmitic acid and linoleic acid and palmitoleic acid were observed to be found in least amounts (Oyase Anthony *et al*., 2016).

An analysis was carried out in order to understand the amount of lipid and cholesterol content present in the tissues of five commercially important species of fish collected from waters around Jaffna Peninsula in Sri Lanka. The average lipid and cholesterol content in the five fishes ranged from 2.63 to 4.41% and 54.2 to 104.5mg/100g respectively. All these values indicate that the fishes have a good nutritional value. This study was important and very useful as these fishes formed a main part of the diet of the people living in the Jaffna Peninsula (S. Sutharshiny *et al*., 2011).

A study was carried out in Turkey where in the fatty acid and cholesterol content in freshwater fishes living in Porsuk dam was determined by Gas-Liquid Chromatography. It was observed that the cholesterol content in the freshwater fishes was found to be low whereas the amount of polyunsaturated fatty acids were high. Due to these values, the fishes had high nutritional value (Muhammed Donmez, 2009).

The proximate composition, amino acid profile and the fatty acid composition was studied in the three different size groups of Commerson's anchovy. The size groups taken were 3-5g, 6-10g and 25-30g. The PUFAs, mineral content and the amino acid content was found to be higher in the small sized anchovies as compared to the other groups. Thus the anchovies were found to be a good source of nutrition for the children and the elderly (T.V. Sankar *et al*., 2013).

A study was carried out in the North-Eastern Atlantic wherein, the protein, moisture, ash and lipid content was estimated in the liver and muscle tissue of 14 commercial marine fishes. The analysis revealed the protein content to be high in all the fish species whereas the fat content was found to be low in all the fish species (Natacha Nogueira *et al*., 2013).

The proximate composition and the mineral content were studied in ten different species of fishes found in the Hel River in North East, India. It was suggested by the authors that the fish species selected have a very good number of proteins,

fats and minerals and therefore were highly recommended for consumption (Arjina Parbin Sarkar *et al*., 2017).

The fatty acid content was investigated in 4 edible species of fish found in the River Hooghly of West Bengal. The fishes chosen for the study were both freshwater and brackish water fishes locally known as Bhola, Ghero, Gule and Vacha. Bhole, Ghero and Vacha were found to be a good source of omega 3 fatty acids whereas the Gule which is a freshwater fish was found to be rich source of omega 6 fatty acids (Nath et al., 2014).

In a study carried out in Sudan, the fatty acid profile was analysed in the Nile fishes by using the Gas chromatography. The predominant fatty acids were found to be palmitic acid, stearic acid, oleic acid, DHA, eladic acid, arachidic acid and palmitoleic acid. Overall, the results of the analysis concluded that all these fish species were a good source of polyunsaturated fatty acids (Elagba Haj Ali Mohamed *et al*., 2011).

In Kerala, another study on the biochemical estimation was carried out on sardine, mackerel and Anchovy by Sumi *et.al*, in 2016. The analysis showed the fishes to be rich in amino acids, EPA and DHA. Also study indicated that the Sardine was found to be a good source of calcium.

In Ratnagiri, the biochemical profile of some of the marine fishes like Pomfret, Indian mackerel, Salmon and King mackerel was studied. Different methods such as Lowry's method for proteins, Lipid estimation by Barnes and Black and

Glycogen by DeZwaan and Zandee were used for the standards. The biomolecules in different organs of the fish were determined such as muscles, liver, kidney and gills. The results indicated the maximum content of protein was present in the muscles of King mackerel, kidney of Salmon and the liver of Indian mackerel whereas the least protein content was present in the gills of all the fishes. The kidney of Pomfret and salmon, and the liver of both King mackerel and Indian mackerel showed maximum amount of lipids whereas the muscles of King mackerel, Pomfret and Indian mackerel showed less amount of lipid content. The glycogen content was observed to be maximum in the kidneys of all four fishes whereas the gills showed the least content of glycogen (Bhilave MP, 2018).

In another analysis, the fatty acid content was determined in the freshwater fishes collected from the fishermen at Kainji Lake dam site. The analysis was carried out by using the GC-MS machine and results showed the presence of a good amount of omega 3 fatty acids present in fish and therefore were recommended for consumption due to its benefits (Robert A. et al., 2014).

OBJECTIVES

Grey mullet and Green chromide are the common fishes that are found on the Goan coastline. Both are declared as the state fish of Goa and Kerala respectively and therefore the local people have to be made aware of their nutritional importance. Not many studies on the nutritional profiling of these fishes have been done. Therefore, the objectives of this study are:

- To prepare a database of nutritive profiles of *Mugil cephalus* (Flathead grey mullet) and *Etroplus suratensis* (Green chromide).
- To compare the seasonal variation of the bioactive compounds, present in both the fish species.

IMPORTANCE OF THE TOPIC RECOGNISED

The study aims to find out the potential nutritive bioactive compounds from Green chromide and Grey mullet which may have importance to medicine, pharmaceuticals, and the industrial sector. It is also important to bring awareness among the local people about the nutritive value of these fishes. This information about the nutrients in fishes will help us to breed them in the captive conditions by supplying them with feed rich in important nutrients.

SPECIES OF FISH STUDIED

1. Green chromide

Scientific name: *Etroplus suratensis* (Bloch, 1790)

Classification

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Cichlidae

Genus: *Etroplus*

Species: *E. suratensis*

Green chromide is the state fish of Kerala. It is a brackish water fish that is found in the states of Goa, Kerala and Odisha. It is fondly eaten in Goa and locally known as Kalunder. The base body colour of the fish is greenish brown and there is a presence of 6 dark vertical bars on the body. Also, numerous body scales have a pearly white central spot and hence it is also commonly known as Pearl spot. It is omnivorous and feeds on aquatic plants, algae, diatoms as well as on molluscs. This fish is known to exhibit nest formation and attentive parental care for the young.

2. **Flathead grey mullet**

Scientific name: *Mugil Cephalus* (Linnaeus, 1758)

Classification

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Mugiliformes

Family: Mugilidae

Genus: *Mugil*

Species: *M. cephalus*

Flat head grey mullet is the state fish of Goa. It is almost widely distributed in many parts of the world. In Goa, locally it is known as Shevto. It has an elongated and silvery body. This fish has the presence of two short separated dorsal fins and forked caudal fin. The anal fin has 8 forked rays in adults and second dorsal and anal fin have scales only on anterior and basal part of the fin. It is herbivorous and known to feed on dead plant matter and detritus. Once these fishes lay their eggs, no parental care is provided.

MATERIALS AND METHODS

MATERIALS AND METHODS

The chemicals used for the carrying out the estimations in this study were concentrated sulphuric acid, sodium-potassium tartarate, sodium carbonate, copper sulphate, Folin's-Ciocalteau reagent, bovine serum albumin, anthrone, glucose, sodium hydroxide, ascorbic acid, ammonium molybdate, absolute alcohol, glycine, stannous chloride, citric acid, tri-sodium citrate, ferric chloride, acetic acid, sodium chloride, di-ethyl ether, chloroform, methanol, perchloric acid, nitric acid, phosphate buffer, cholesterol standard solution and ethyl acetate.

The glass wares utilised for the purpose of estimations were test tubes, centrifuge tubes, micropipettes, measuring cylinder, test tube stand, glass rods, beakers, desiccator, crucibles and separating funnel.

Spectrophotometer, atomic absorption spectrophotometer, gas chromatography – mass spectrophotometer, vortex, centrifuge, water bath and sonicator were the instruments used for the estimation.

SAMPLING OF THE FISH

Green chromide and Grey mullet were both obtained from the sluice gate located at Neugi- Nagar, Goa. They were obtained from the local fishermen. The duration of the study was from June 2022 to January 2023. The fish specimens were brought to the laboratory and dissected, after which the muscle

tissue was obtained. This muscle tissue was used for the estimation of various parameters. The different parameters estimated were crude protein, carbohydrates, fatty acids, cholesterol, amino acids, ash, moisture, vitamins and minerals.

METHODOLOGY

EXTRACTION PROCEDURE

The fish specimens were cleaned and then dissected. About 5gms of the muscle tissue was extracted. This was homogenised with 10 ml of phosphate buffer. The homogenate was stored in blue capped centrifuge tubes and 1/3 of it was used for the estimation of carbohydrates. Equal amount of perchloric acid was added to the remaining homogenate. This was then centrifuged at 4000 rpm for 10 minutes. The supernatant thus obtained was used for the estimation of amino acids. After this to the remaining residue of the muscle tissue about 4ml of chloroform and 2ml of methanol was added. This mixture was then again centrifuged at 4000 rpm for 10 minutes. The supernatant obtained hence was used for the cholesterol estimation. After this again a volume of 5ml of perchloric acid was added to the remaining muscle tissue residue and centrifugation was done at 4000 rpm for 10 minutes. The supernatant obtained was discarded. After this 2ml of 1N NaOH was added to the remaining residue and this was kept in a boiling water bath for half an hour to get a clear solution. The clear solution was then used for the estimation of proteins.

1. **PROTEIN TEST (**Lowry *et al*., 1951)

Principle

The peptide nitrogen reacts with the copper ions under alkaline conditions and copper catalyses the oxidation of the aromatic acids and converts the Folin's Ciocalteau phosphomolybdic phosphotungstic acid to heteromolybdenum blue. The blue colour obtained is read at 660 nm.

Reagent preparation

1. **Lowry's reagent**

a. Solution A - 4g of sodium carbonate was added in 100 ml of distilled water.

b. Solution B - 0.1g of copper sulphate was added in 5ml of distilled water.

c. Solution C – 0.2g of Sodium-Potassium Tartrate was added in 5 ml of distilled water

98 ml of solution A + 1ml of solution B + 1ml of solution C was mixed together to prepare the Lowry's reagent.

2. **Folin's Ciocalteau reagent**

10 ml of Folin's reagent was added in 10 ml of distilled water to make 1:1 Folin's solution.

Estimation

The BSA standard solution was added in serially increasing concentrations in 6 of the test tubes. This was then diluted up to 1 ml with distilled water. Then 5ml of Lowry's reagent was added and incubation was done for up to 10

minutes. This was followed by the addition of 0.5ml of Folin's reagent and again incubation for 10 minutes. Finally, the absorbance was read against the blank at 660 nm. For the sample, 1ml of the extracted fish tissue sample was taken and in that 5ml of Lowry's reagent was added and this was followed by incubation for 10 minutes. Then 0.5ml of Folin's reagent was added and again incubation was done for about 10 minutes. The absorbance was then finally read at 660 nm. Concentration of the proteins was determined from the unknown OD by using the standard curve.

2. **CARBOHYDRATE TEST (**Hedge and Hofrreiter, 1962)

Principle

Formation of furfural takes place when carbohydrates are dehydrated with concentrated sulphuric acid. Then condensation of furfural with that of anthrone takes place in order to form a green coloured complex which is measured with the help of the spectrophotometer at 620 nm.

Preparation of reagents

Anthrone reagent: 0.2g of anthrone powder was added in 100 ml of concentrated sulphuric acid.

Estimation

Standard solution of glucose was added in a serially increasing manner in 6 test tubes (0.2-1ml). This was diluted up to 1ml by using distilled water. The blank test tube contained just 1ml of distilled water. After this 5ml of anthrone

reagent was added to test tubes and then they were kept in a boiling water bath for about 20 minutes. The OD was then read at 620 nm. For the sample, 1ml of the extracted fish sample was taken and then 5ml of anthrone reagent was added. This was followed by incubation in a boiling water bath for 20 minutes and finally absorbance was read against the blank at 620 nm.

3. <u>ASH AND MOISTURE CONTENT</u> (AOAC, 2000)

Small crucibles were obtained and then the weight of the empty crucible was taken and this was recorded as (1). 1gm of the fish muscle tissue was weighed separately and put into the crucible. The crucible with the fish tissue was then weighed and this weight was noted as (2). The crucible containing the fish tissue was then placed in the hot air oven at a temperature of 105 degree Celsius for about an hour. The crucible was then weighted after cooling and this weight was recorded as (3). The crucibles were placed in the muffle furnace after this for 1 hour at 550 degrees Celsius for the ash content. Then the crucibles were cooled and weighed and this weight was noted as (4).

<u>Calculations for ash and moisture</u>

Wet weight (A) = (2) – (1)

Dry weight (B) = (3) – (1)

Moisture = [A – B / A] X 100

C = (4) – (1) [required for calculating ash]

Ash = [C/A] X 100

4. **CHOLESTEROL TEST** (Zak and Ressler, 1955)

Principle

Cholesterol is dehydrated on treatment with concentrated sulphuric acid and there is a formation of a red coloured complex due to the catalytic action of the ferric ions which is measured at 550nm.

Preparation of reagents

$FeCl_3$ – Acid mixture – 0.0125g of $FeCl_3$ powder was dissolved in orthophosphoric acid and this mixture was then added in 50 ml of concentrated sulphuric acid.

Estimation

Cholesterol standard solution was added in a serially increasing manner in 7 test tubes. A blank was also maintained containing 3ml acetic acid instead of the standard. The volume in the tubes was made up to 3ml using acetic acid. Then about 2ml of ferric chloride was added in each of the test tubes and they were incubated at room temperature for 10 minutes followed by which the optical density was measured by using a spectrophotometer at 550 nm. For the sample, the cholesterol extract obtained was used in the required quantity and then its volume was made up to 3 by using acetic acid. Then 2ml of ferric chloride solution was added and there after incubation was done at room temperature for 10 minutes. Finally the OD was read at 550 nm.

5. **VITAMIN C TEST** (Rajput *et al*., 2011)

Preparation of reagents

1. 10% sulphuric acid – 5ml of sulphuric acid was added in 50 ml of distilled water.

2. 10% ammonium molybdate – This was prepared by the addition of 5g of ammonium molybdate in 50 ml of distilled water.

Fish tissue processing

0.1g of the muscle tissue of the fish was weighed and then homogenised with 2 ml of distilled water. This homogenate was collected in a centrifuge tube and in that about 2ml of perchloric acid was added and then the tubes were centrifuged. The supernatant obtained after centrifugation was used for the analysis of vitamin C.

Estimation

Standard solution was added in a serially increasing manner in 50 ml capacity centrifuge tubes. 2ml of distilled water was added in the blank tube and similarly for the tubes containing the unknown sample, 2ml of the supernatant obtained was used. After this, 2ml of 10% sulphuric acid, followed by 5ml of 10% ammonium molybdate was added in all the tubes. The contents present in the tubes were mixed thoroughly by using a vortex mixer. After this, the tubes were kept for incubation at room temperature for 50 minutes. After the incubation period the volume of the tubes, was made up to 25 ml by adding distilled water. Finally, the absorbance was read against the blank at 540 nm. The standard

curve was then constructed using excel and used to estimate the concentration of vitamin C in the fish tissues.

6. **ESTIMATION OF MINERALS** (AOAC, 2000)

Fish tissue digestion and processing

0.2 g of the muscle tissue of the fish was weighed and taken into a test tube. To this test tube, 4 ml of mixture of nitric acid and perchloric acid was added in the concentration of 3:1. This was kept for digestion for about a day. After this, the clear solution was obtained. This clear solution was filtered through the Whatmann's filter paper. 1ml of the filtered solution was taken and made up to 100 ml by dilution with distilled water. This diluted solution was stored in 2, 50 ml capacity centrifuge tubes to be analysed for zinc using atomic absorption spectrophotometer and for iron, sodium and potassium using flame photometer.

7. **ESTIMATION OF AMINO ACIDS (**Mahesha H B, 2012)

Principle

Ninhydrin which is a very powerful oxidising agent results in decarboxylation of the alpha amino acids and forms a bluish-purple product called as Ruhemman's purple which can be measured colorimetrically at 570nm.

Preparation of Reagents

Ninhydrin solution: 1g of ninhydrin powder was mixed with 25ml of ethanol and 40mg of stannous chloride was mixed with 25ml of citrate buffer. Both the solutions were mixed to form the ninhydrin solution.

Citrate buffer: 0.21 g of citric acid and 0.29 g of sodium citrate were dissolved in 10ml of distilled water separately. 7.6ml of citric acid and 7.4ml of sodium citrate was mixed then the volume was made to 30ml with distilled water. The pH was then adjusted to 5.

Estimation

The standard amino acid solution was pipetted in 6 different test tubes in a serially increasing manner. 1ml distilled water was added in the blank test tube. For the unknown test tube, 1ml of the fish sample extract was taken. Then, to all the test tubes, 1ml of ninhydrin was added. Followed by this the tubes, were incubated in the boiling water bath for 15 minutes. After the incubation 5ml of the citrate buffer was added in all the test tubes and then the tubes were incubated at room temperature for 10 minutes. Finally, the absorbance was taken against the blank at 570 nm.

8. **FATTY ACID PROFILES** (O' Fallon *et al*., 2007)

Processing of the fish sample:

1g of the fish muscle tissue was weighed and then homogenized in 10 ml of 2:1 chloroform- methanol mixture using a mortar and pestle. The homogenate was collected in a centrifuge tube and then centrifuged at 4000 rpm for 10

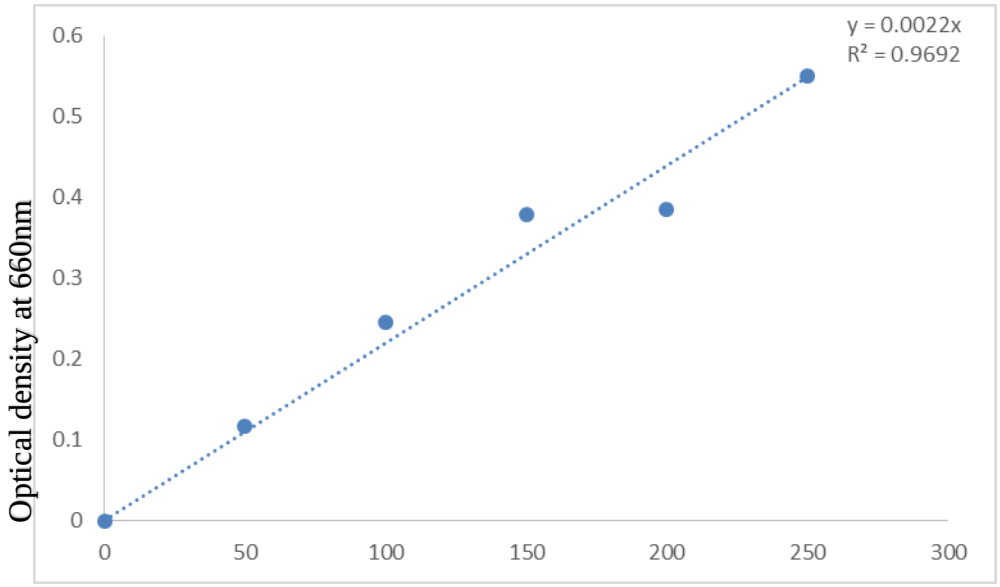
minutes. The supernatant was then collected in a beaker and then again 5ml of diethyl ether was added to the remaining residue. This was again centrifuged at 4000 rpm for 10 minutes. This was followed by addition of the new supernatant to the earlier supernatant. After this to the mixture of both the new and the old supernatant about 2.5ml of 10% NaCl was added. This mixture was then kept in the separating funnel overnight for phase separation.

Extraction:

The top clear layer was taken and then processed further. It was then put in the sonicator bath for 15 minutes. From this 0.5 ml was taken in test tubes and in that 0.7ml of 10N KOH and 5.3ml of methanol was added. This was followed by keeping the tubes in the water bath for 2 hours at 55 degrees Celsius. The contents of the tubes were mixed thoroughly by using a vortex mixer after every 20 minutes. The tubes were then cooled and 0.58 ml of 24N sulphuric acid was added from the sides. A white precipitate was obtained which was mixed thoroughly using a vortex mixer. Again, the tubes were kept in the water bath for 2 hours at 55 degrees Celsius. The tubes were then cooled and in that 3 ml of ethyl acetate was added. The tubes were then vortexed for 5 minutes. The clear top layer was obtained and then collected in Teflon vials. This volume was reduced again by the use of nitrogen gas and was then analysed by using GC-MS analysis.

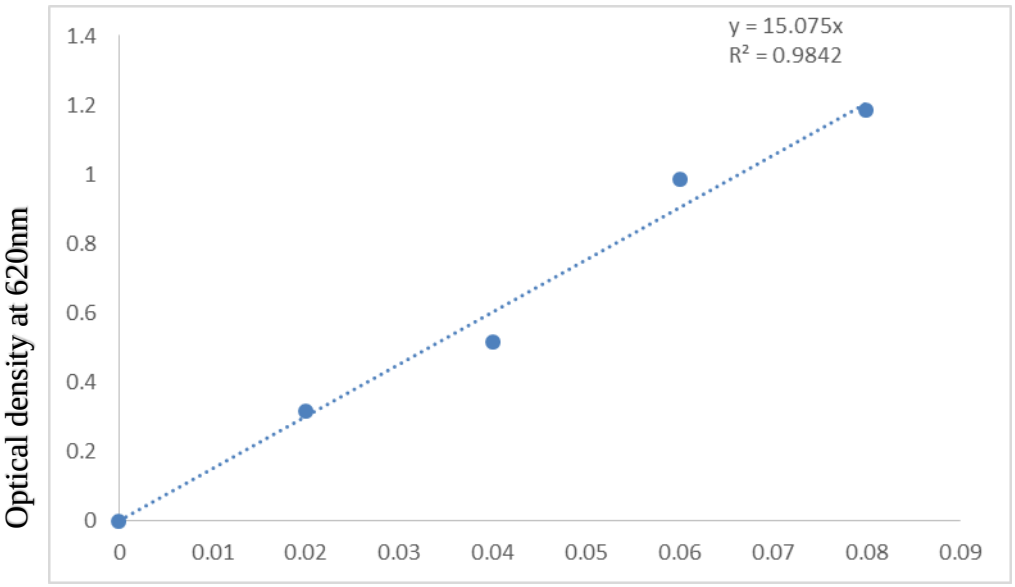
STANDARD CURVES

STANDARD CURVE FOR PROTEIN



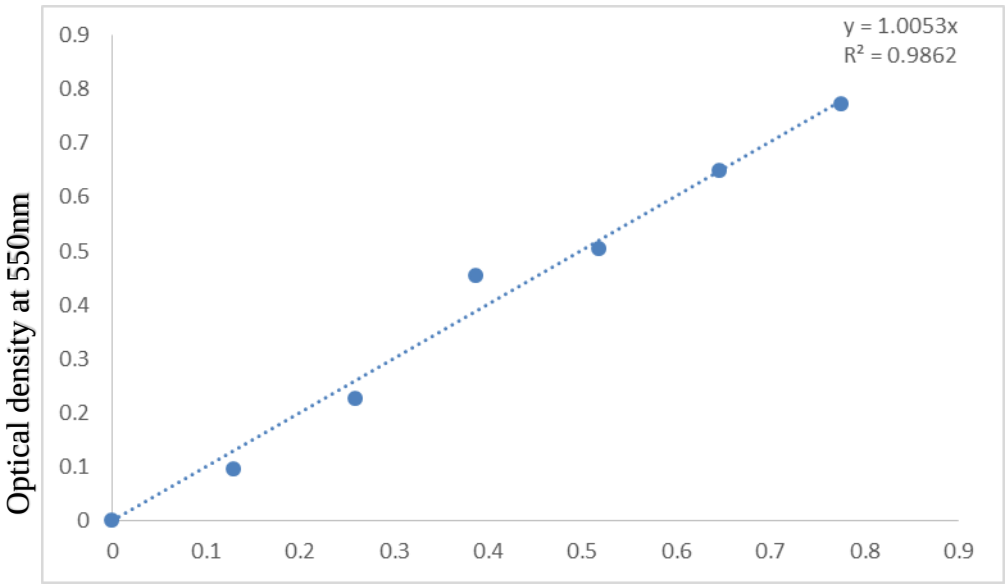
Concentration of BSA in µg/ml

STANDARD CURVE FOR CARBOHYDRATES



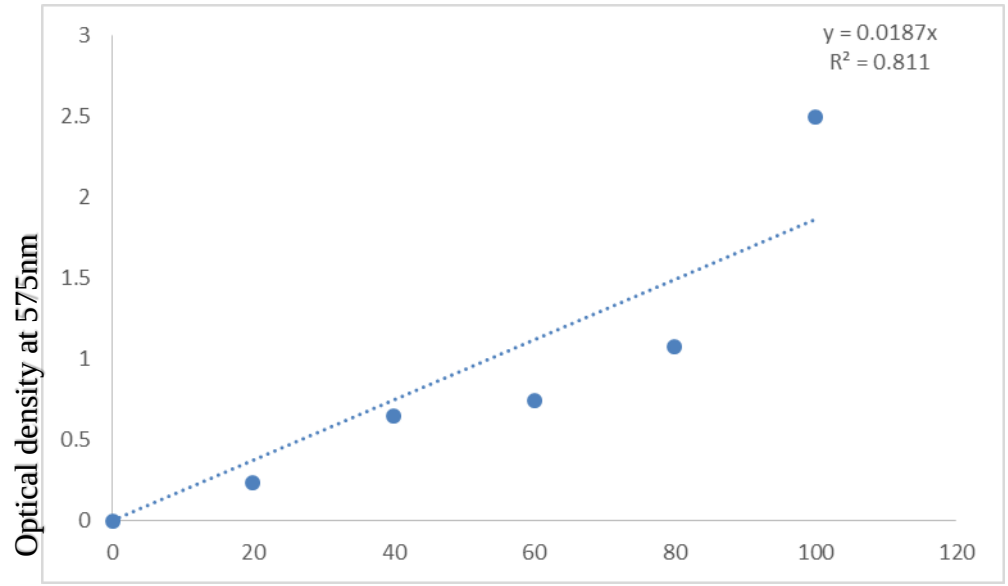
Concentration of glucose in mg/ml

STANDARD CURVE FOR CHOLESTEROL



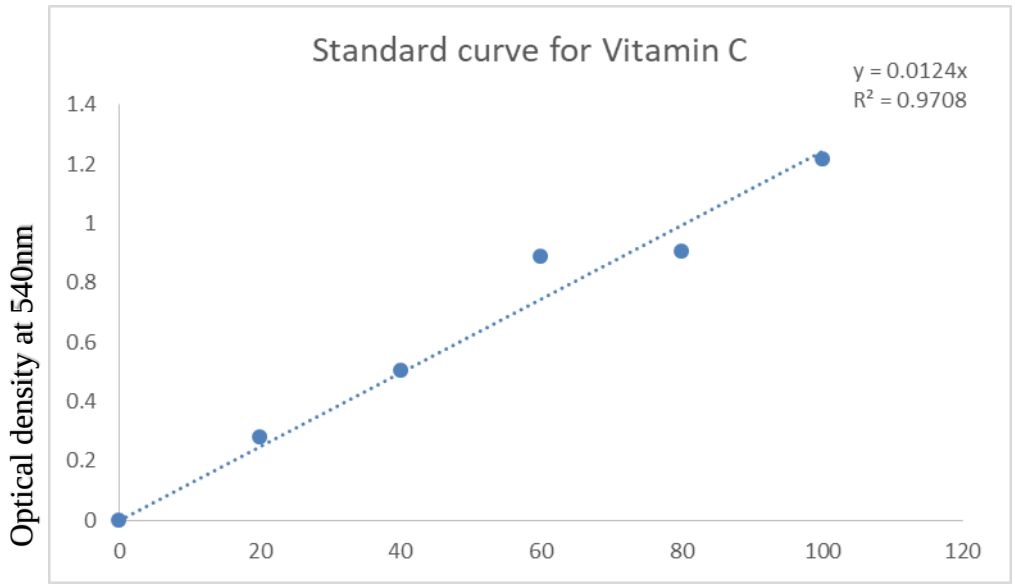
Concentration of Cholesterol in μ moles/ml

STANDARD CURVE FOR AMINO-ACIDS

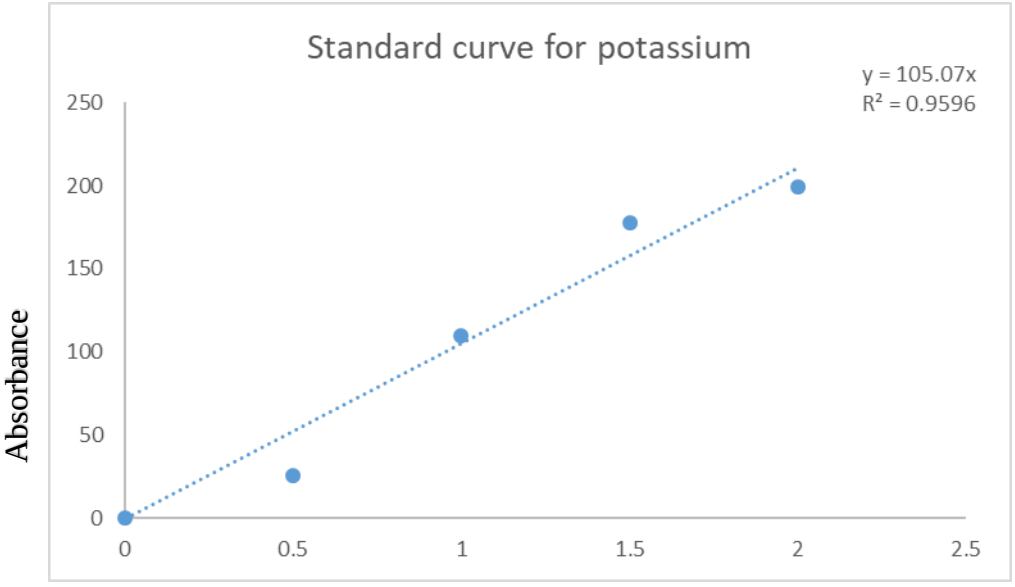


Concentration of the glycine stock solution in μg/ml

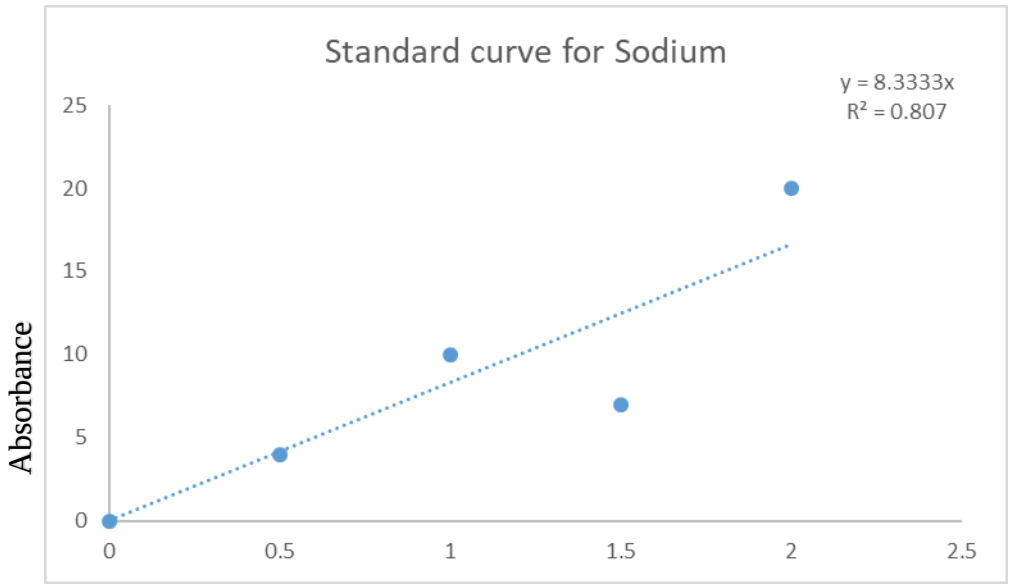
STANDARD CURVE FOR VITAMIN C



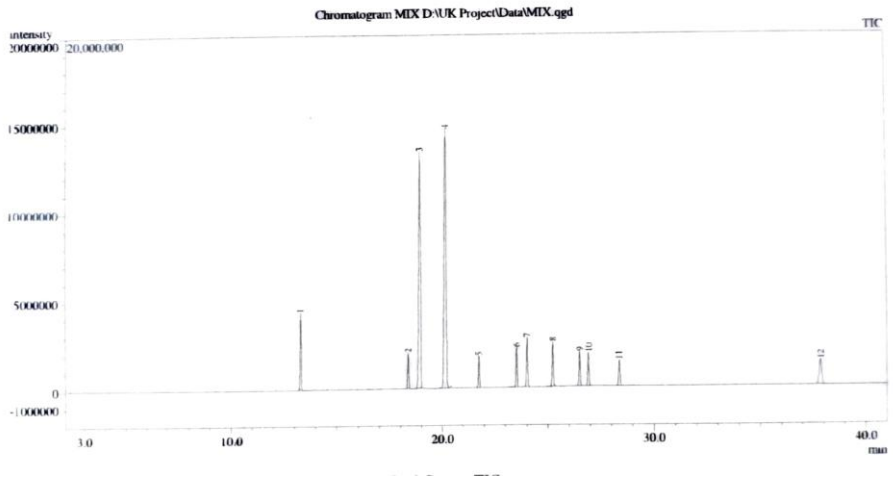
STANDARD CURVE FOR POTASSIUM



STANDARD CURVE FOR SODIUM



STANDARD CURVE FOR FATTY ACIDS



PLATES

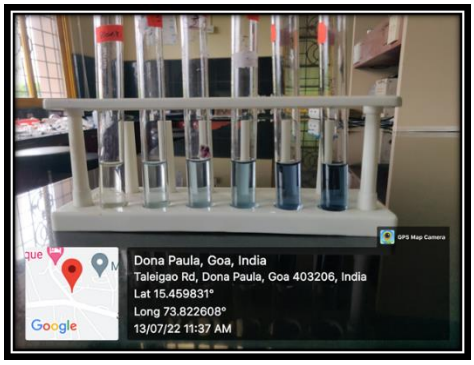


Plate 1: Standard test for proteins

Plate 2: Standard test for carbohydrates

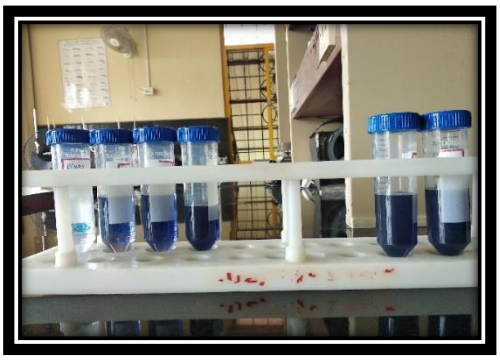


Plate 3:Vitamin C standard test

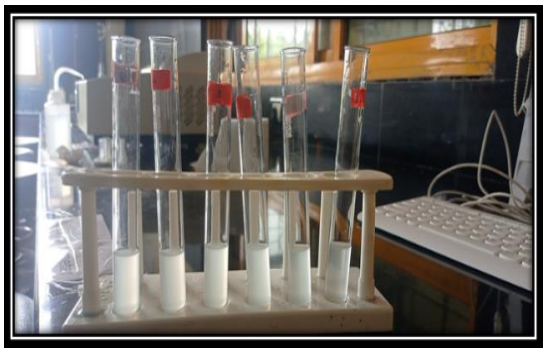


Plate 4: Standard test for amino acids

Plate 5: Phase separation in fatty acid test

Plate 6: Ash content

TABLES

OBSERVATION TABLE FOR PROTEIN CONCENTRATION PRESENT IN THE MUSCLE TISSUE OF GREEN CHROMIDE AND GREY MULLET

Months	Green chromide protein concentration in µg/ml	Grey mullet protein concentration in µg/ml
June	912.54 ±252.73	1061.45 ±260.05
July	1091.13 ±394.24	752.46 ±432.77
August	965.17 ±369.13	897.99 ±357.18
September	416.35 ±163.81	507.436 ±239.83
October	442.42 ±253.14	819.38 ±232.42
November	474.54 ±61.57	660.75 ±130.69
December	325.75 ±63.39	783.33 ±201.58
January	1204.993 ±29.98	966.66 ±242.92
P value	0.106	0.106
F value	0.106	0.106

OBSERVATION TABLE FOR CARBOHYDRATE CONCENTRATION PRESENT IN THE MUSCLE TISSUE OF GREEN CHROMIDE AND GREY MULLET

Months	Green chromide carbohydrate concentration in µg /ml	Grey mullet carbohydrate concentration in µg /ml
June	170.79 ± 14.07	145.00 ±55.44
July	187.58 ± 7.00	162.96 ±34.77
August	168.73 ± 41.77	128.73 ±28.26
September	190.16 ± 6.20	168.88 ±26.41
October	126.62 ± 32.73	182.32 ±7.76
November	115.72 ± 25.24	187.47 ±1.76
December	110.75 ± 12.05	187.74 ±1.76
January	47.44 ± 5.40	134.83 ±40.06
P value	0.94	0.94
F value	0.94	0.94

OBSERVATION TABLE FOR AMINO-ACID CONCENTRATION PRESENT IN THE MUSCLE TISSUE OF GREEN CHROMIDE AND GREY MULLET

Months	Green chromide amino-acid concentration in µg/ml	Grey mullet amino-acid concentration in µg/ml
June	124.71 ±26.40	110.53 ±49.55
July	120.54 ±42.47	89.98 ±40.06
August	99.18 ±42.23	66.54 ±25.84
September	110.78 ±35.16	100.51 ±35.13
October	55.05 ±5.19	77.77 ±4.07
November	54.00 ±5.96	105.25 ±44.04
December	51.47 ±0.93	57.91 ±2.55
January	60.85 ±2.31	81.60 ±39.34
P value	0.25	0.25
F value	0.25	0.25

OBSERVATION TABLE FOR CHOLESTEROL CONCENTRATION PRESENT IN THE MUSCLE TISSUE OF GREEN CHROMIDE AND GREY MULLET

Months	Green chromide cholesterol concentration in µg/ml	Grey mullet cholesterol concentration in µg/ml
June	0.438 ±0.09	0.454 ±0.03
July	0.456 ±0.13	0.448 ±0.06
August	0.452 ±0.05	0.474 ±0.05
September	0.320 ±0.03	0.358 ±0.05
October	0.023 ±0.01	0.103 ±0.03
November	0.109 ±0.13	0.099 ±0.02
December	0.074 ±0.02	0.102 ±0.06
January	0.155 ±0.06	0.108 ±0.03
P value	0.00002	0.00002
F value	131.91	131.91

OBSERVATION TABLE FOR ASH AND MOISTURE CONTENT PRESENT IN THE MUSCLE TISSUE OF GREEN CHROMIDE AND GREY MULLET

Months	Green chromide moisture content in percent	Green chromide ash content in percent	Grey mullet moisture content in percent	Grey mullet ash content in percent
June	78.54% ±14.19	4.66% ±2.76	71.75% ±15.20	4.79% ±2.36
July	74.97% ±14.19	4.8% ±2.76	62.5% ±15.20	8.2% ±2.36
August	73.7% ±14.19	5.45% ±2.76	74.12% ±15.20	0.68% ±2.36
September	46.07% ±14.19	1.24% ±2.76	41.5% ±15.20	3.97% ±2.36
October	51% ±14.19	9.4% ±2.76	57.66% ±15.20	3.2% ±2.36
November	80.39% ±14.19	1% ±2.76	41% ±15.20	1.9% ±2.36
December	72.77% ±14.19	2.03% ±2.76	33% ±15.20	1.7% ±2.36
January	85.87% ±14.19	3.2% ±2.76	47% ±15.20	4.8% ±2.36
P value	0.76	0.79	0.022	0.79
F value	0.76	0.79	0.76	0.79

OBSERVATION TABLE FOR VITAMIN C CONCENTRATION PRESENT IN THE MUSCLE TISSUE OF GREEN CHROMIDE AND GREY MULLET

Months	Green chromide vitamin C concentration in µg/ml	Grey mullet vitamin C concentration in µg/ml
June	6.370 ±1.92	3.951 ±2.11
July	6.048 ±1.92	2.983 ±2.11
August	6.774 ±1.92	4.838 ±2.11
September	5.645 ±1.92	3.951 ±2.11
October	7.903 ±1.92	8.225 ±2.11
November	4.354 ±1.92	7.177 ±2.11
December	10 ±1.92	7.096 ±2.11
January	4.032 ±1.92	2.661 ±2.11
P value	0.17	0.79
F value	2.30	0.79

OBSERVATION TABLE FOR POTASSIUM CONCENTRATION PRESENT IN THE MUSCLE TISSUE OF GREEN CHROMIDE AND GREY MULLET

Months	Concentration of potassium in Green chromide in ppm	Concentration of potassium in Grey mullet in ppm
June	0.87 ±0.28	0.79 ±0.10
July	0.72 ±0.28	0.61 ±0.10
August	0.39 ±0.28	0.85 ±0.10
September	0.62 ±0.28	0.69 ±0.10
October	0.43 ±0.28	0.72 ±0.10
November	0.418 ±0.28	0.61 ±0.10
December	1.199 ±0.28	0.78 ±0.10
January	0.42 ±0.28	0.57 ±0.10
P value	0.43	0.43
F value	0.70	0.70

OBSERVATION TABLE FOR SODIUM CONCENTRATION PRESENT IN THE MUSCLE TISSUE OF GREEN CHROMIDE AND GREY MULLET

Months	Concentration of sodium in Green chromide in ppm	Concentration of sodium in Grey mullet in ppm
June	3.60 ±17.47	3.60 ±4.57
July	1.20 ±17.47	2.40 ±4.57
August	2.40 ±17.47	2.40 ±4.57
September	2.40 ±17.47	15.60 ±4.57
October	1.20 ±17.47	2.40 ±4.57
November	2.40 ±17.47	2.40 ±4.57
December	51.60 ±17.47	3.60 ±4.57
January	2.40 ±17.47	2.40 ±4.57
P value	0.88	0.88
F value	0.88	0.88

OBSERVATION TABLE FOR IRON AND ZINC CONCENTRATION PRESENT IN THE MUSCLE TISSUE OF GREEN CHROMIDE AND GREY MULLET.

Months	Concentration of iron in Green chromide in ppm	Concentration of iron in Grey mullet in ppm	Concentration of zinc in Green chromide in ppm	Concentration of zinc in Grey mullet in ppm
June	0.2 ±0.10	0.06 ±0.12	0.07 ±0.02	0.03 ±0.01
July	0.18 ±0.10	0.04 ±0.12	0.04 ±0.02	0.01 ±0.01
August	0.01 ±0.10	0.26 ±0.12	0.02 ±0.02	0.02 ±0.01
P value	0.11	0.11	0.59	0.59
F value	30.81	30.81	0.59	0.59

OBSERVATION TABLE FOR FATTY ACIDS PRESENT IN THE MUSCLE TISSUE OF GREEN CHROMIDE AND GREY MULLET

Green chromide	**Grey mullet**
Heptasiloxane ($C_{14}H_{44}O_6Si$)	Hexadecanoic acid/Palmitic acids ($C_{17}H_{34}O_2$)
Trisiloxane ($C_{18}H_{22}O_2Si$)	Benzene-dicarboxylic acid/Pthalic acid ($C_{16}H_{24}O_4$)
Cyclo-Octasiloxane ($C_{16}H_{48}O_8Si$)	Palmitoleic acid ($C_{16}H_{30}O_2$)
Cyclononasiloxane ($C_{18}H_{54}O_9Si$)	Hexadecatetranoic acid ($C_{16}H_{24}O_2$)
Salbutamol tritbdms ($C_{31}H_{63}NO_3$)	Oleic acid (C18H34O2)
Oleic acid ($C_{18}H_{34}O_2$)	Eicosapentanoic acid/EPA ($C_{20}H_{30}O_2$)
Linoleic acid ($C_{18}H_{32}O_2$)	9-Octadecanoic acid/Stearic acid ($C_{18}H_{34}O_2$)
Linoleic acid ($C_{18}H_{32}O_2$)	Linoleic acid ($C_{18}H_{32}O_2$)
	Linolenic acid ($C_{18}H_{30}O_2$)
	Pentadecanoic acid ($C_{15}H_{32}O_2$)
	Heptadecanoic acid ($C_{18}H_{34}O_2$)
	Methyl Stearase ($C_{19}H_{38}O_2$)
	5,8,11,14- eicosatetraenoic acid ($C_{20}H_{32}O_2$)

FIGURES

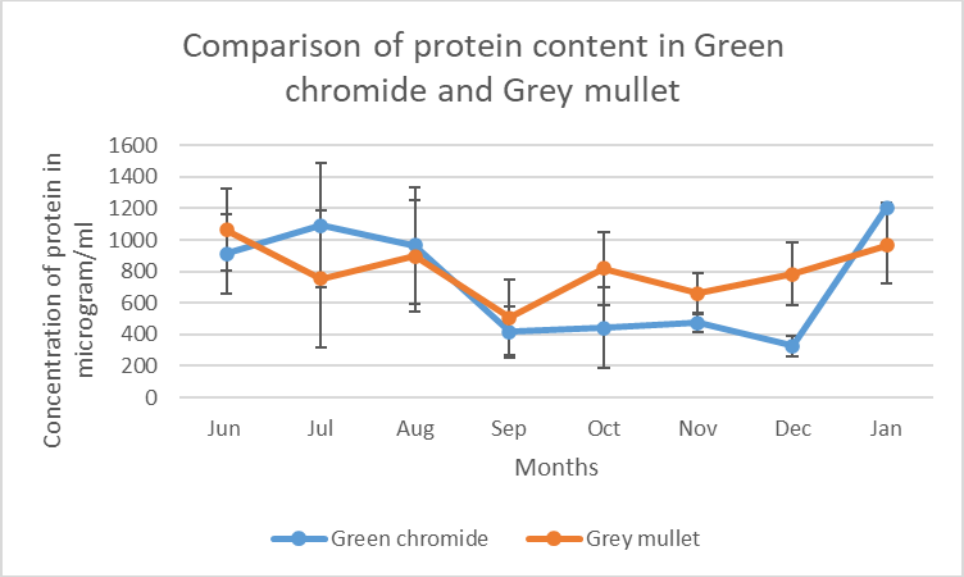


Figure 1: Variation of Proteins in Green chromide and Grey mullet along the months of June 2022 to January 2023.

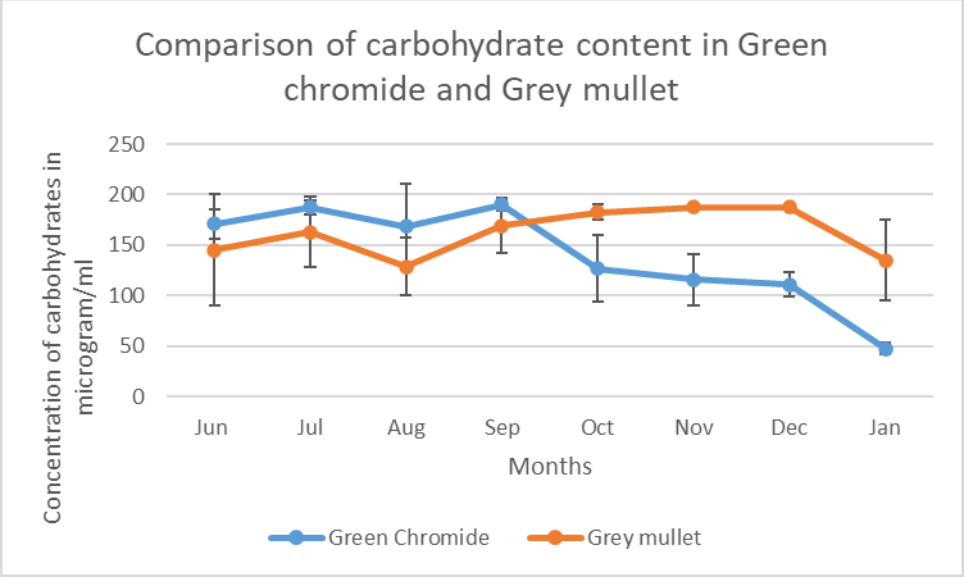


Figure 2: Variation of carbohydrates in Green chromide and Grey mullet along the months of June 2022 to January 2023.

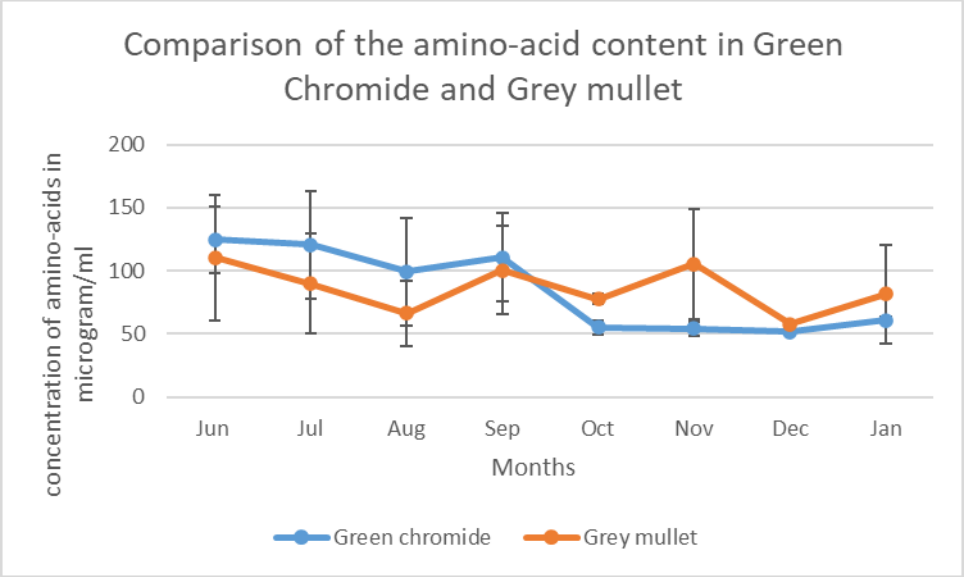


Figure 3: Variation of amino-acids in Green chromide and Grey mullet along the months of June 2022 to January 2023.

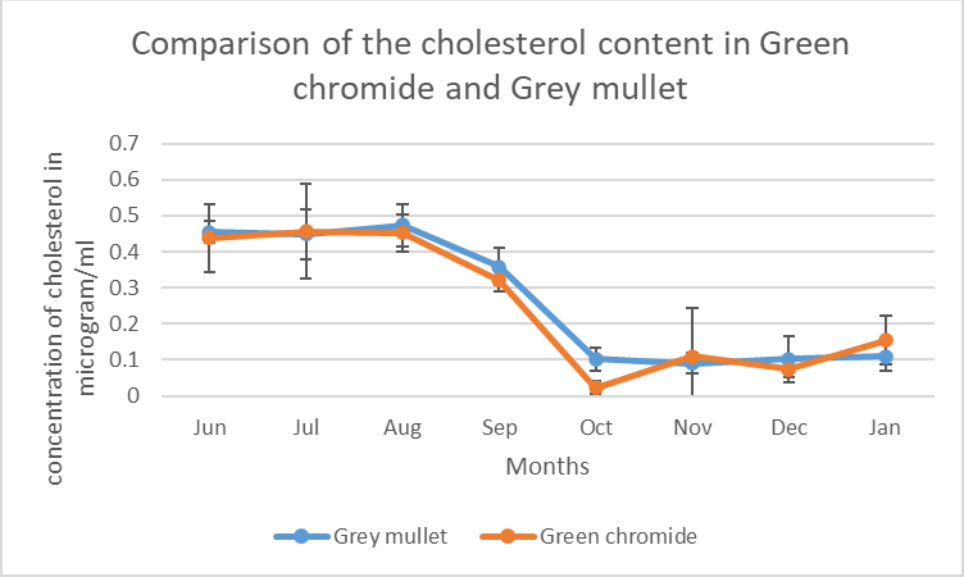


Figure 4: Variation of cholesterol in Green chromide and Grey mullet along the months of June 2022 to January 2023.

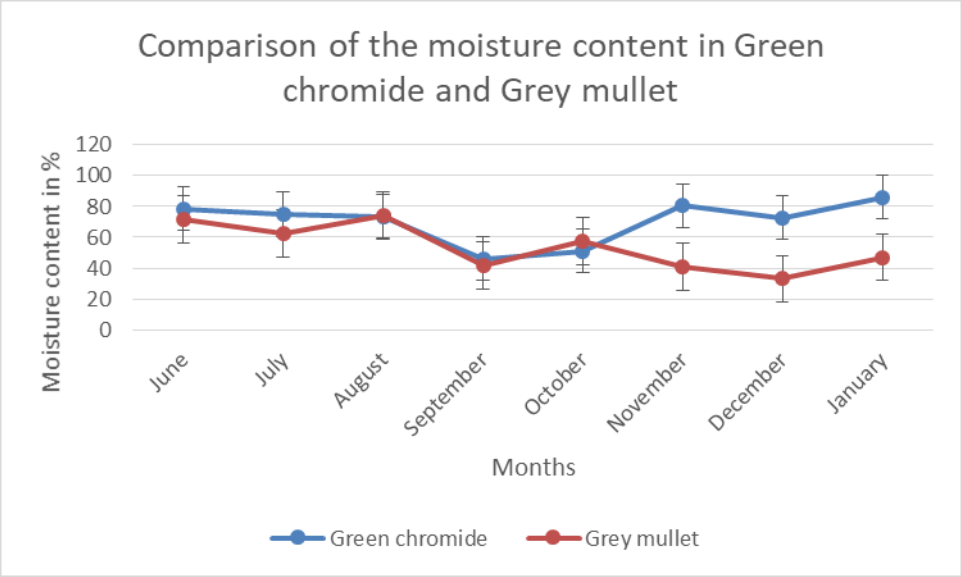


Figure 5: Variation of moisture content in Green chromide and Grey mullet along the months of June 2022 to January 2023.

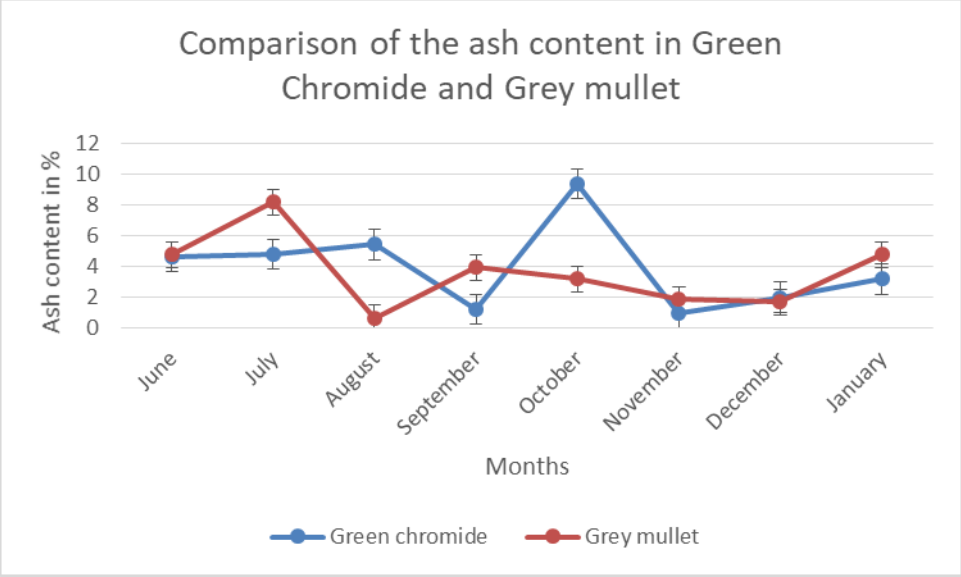


Figure 6: Variation of ash content in Green chromide and Grey mullet along the months of June 2022 to January 2023.

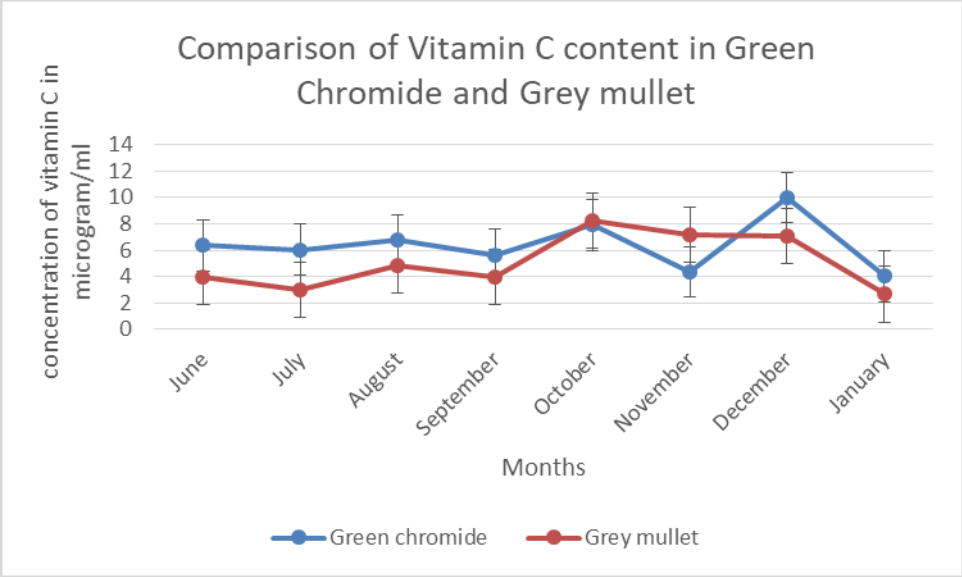


Figure 7: Variation of vitamin C content in Green chromide and Grey mullet along the months of June 2022 to January 2023.

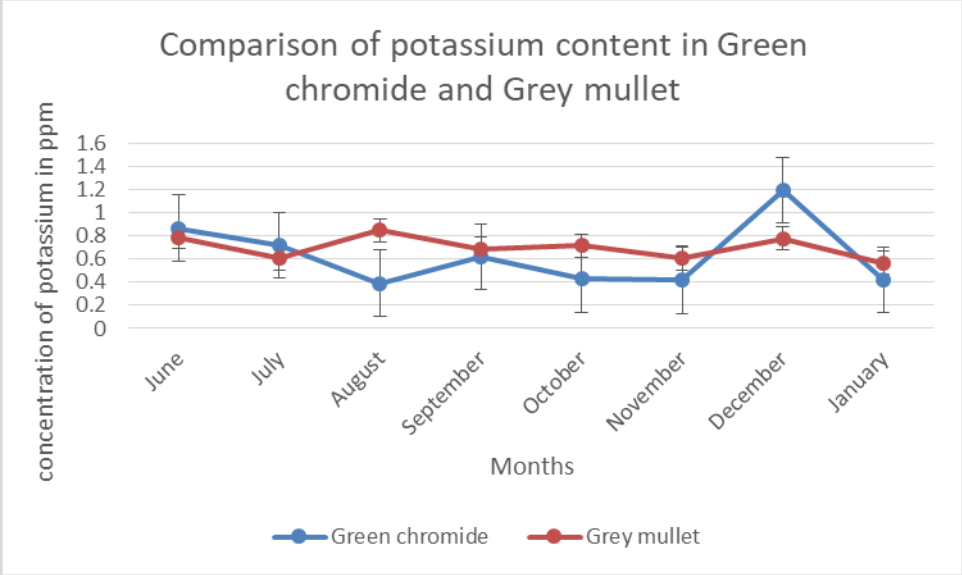


Figure 8: Variation of potassium content in Green chromide and Grey mullet along the months of June 2022 to January 2023.

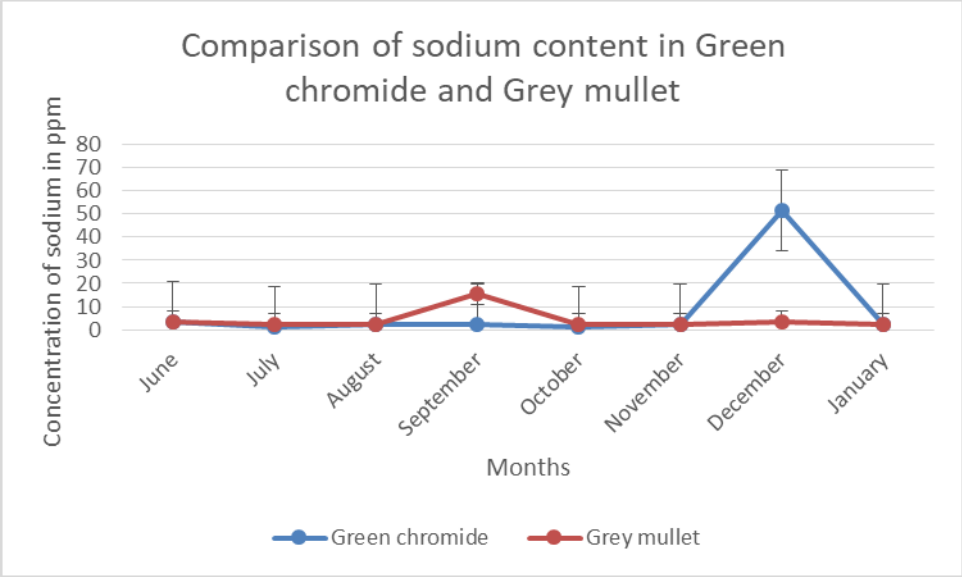


Figure 9: Variation of sodium content in Green chromide and Grey mullet along the months of June 2022 to January 2023.

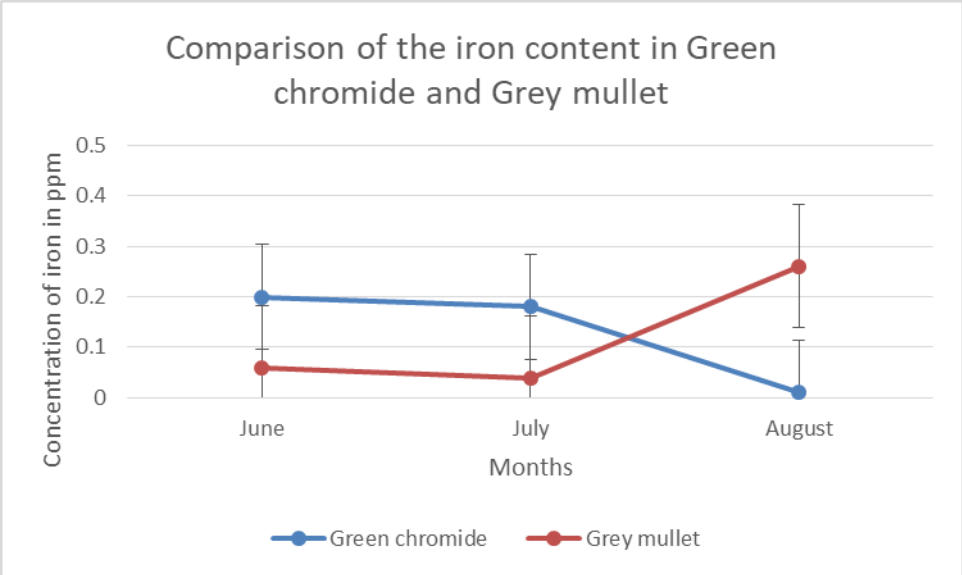


Figure 10: Variation of iron content in Green chromide and Grey mullet along the months of June 2022 to August 2022.

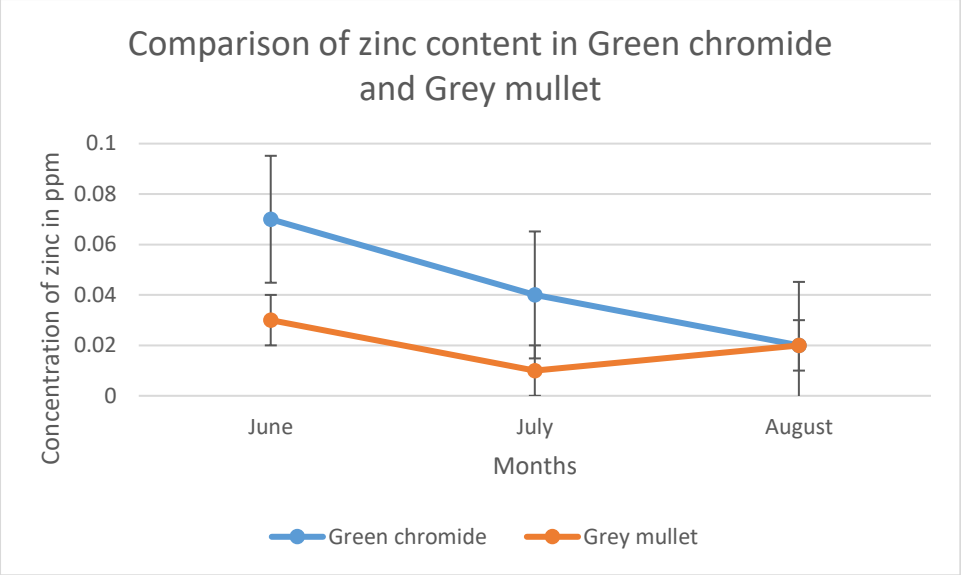


Figure 11: Variation of zinc content in Green chromide and Grey mullet along the months of June 2022 to August 2022.

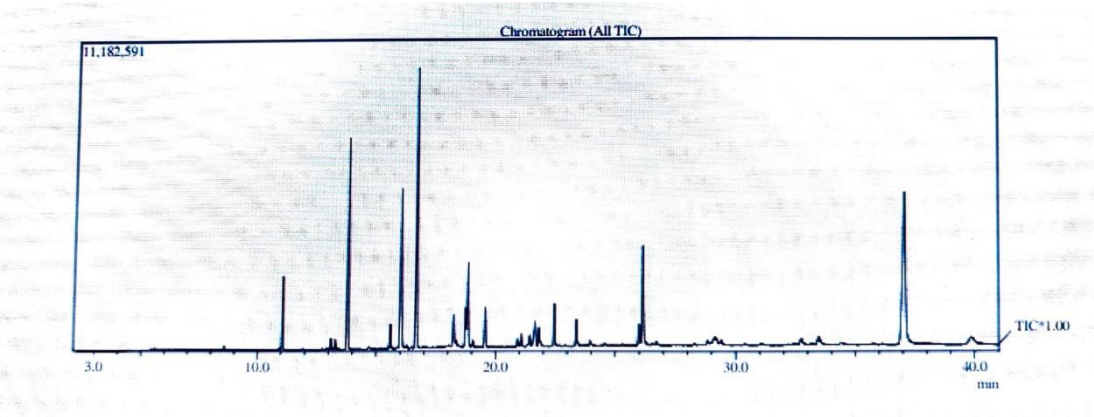


Figure 12: Graph observed for Grey mullet by GC-MS.

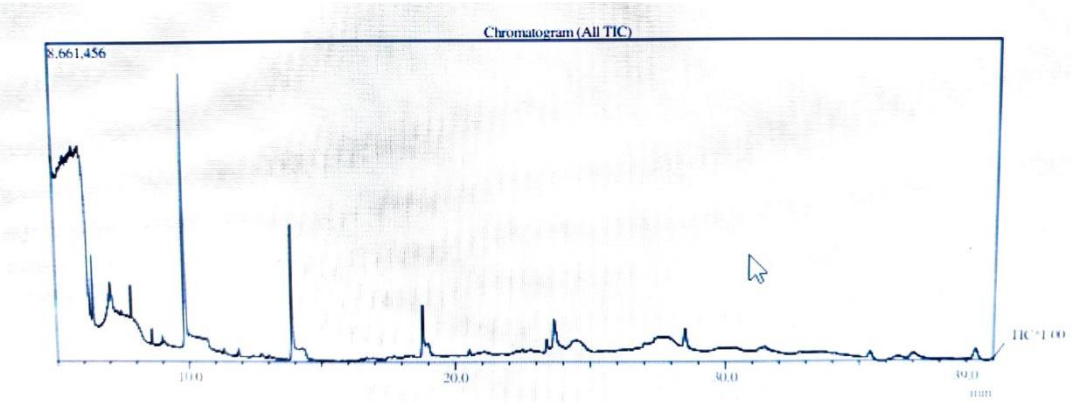


Figure 13: Graph observed for Green chromide by GC-MS.

RESULTS

RESULTS

Proximate composition of the muscle tissue in Green chromide and Grey mullet.

Proteins

In Green chromide, since $p>0.05$, it does not show any significant variation across the months studied.

In Grey mullet, since $p>0.05$, there is no clear distinction among the protein content across the months studied.

In Green chromide, protein concentration was found to be the highest in the month of January and in Grey mullet, the highest value was observed in the month of June. A decrease in the value of the concentration of protein was observed for both the fishes in the months of September, October, November and December. A low level of protein was observed for Green chromide in the month of December and for Grey mullet it was in the month of September. (Figure1)

Carbohydrates

In Green chromide, since $p>0.05$, it shows no significant variation across the study period.

In Grey mullet, since $p>0.05$, there is no clear significance in the carbohydrate content across the months studied.

The highest concentration of carbohydrates was found to be observed in Grey mullet in the month of December. The carbohydrate concentrations for Grey mullet show an increase from September onwards whereas in Green chromide,

it shows a decrease in the concentration values from October onwards. (Figure 2)

Amino-acids

In Green chromide, since $p>0.05$, no significant variation was seen across the study period.

In Grey mullet, since $p>0.05$, there is no difference in the amino acid values observed across the months.

For both the species of fish studied, the highest concentration of amino acids was found to occur in the month of June and the lowest was found to occur in the month of December. Almost similar levels of amino acids were found to occur in both the fishes in all the months studied. (Figure 3)

Cholesterol

In Green chromide, since $p<0.05$, clear variation was observed in the cholesterol content across the months studied.

In Grey mullet, since $p<0.05$, significant variation was seen in the cholesterol content across the study period.

In both species of fish, the cholesterol levels were found to be lower than the proteins, carbohydrates and amino-acids. The levels observed were similar in both the fishes and showed a decreasing trend from the months of September to January. However, the highest levels for Green chromide was observed in the month of July and for Grey mullet it was in the month of August. (Figure 4

Moisture content

In Green chromide, since $p>0.05$, there is no clear variation in the moisture content across the study period.

In Grey mullet, since $p<0.05$, significant difference in the moisture content was observed across the study period.

The moisture content showed variable levels in both the species of fish studied. The highest level of moisture was found to be observed in Green chromide in the month of January whereas for Grey mullet, the highest level was observed in the month of June. The moisture content was found to be low in the month of September for Green chromide and for Grey mullet it was found to be low in the month of December. Overall, after the comparison of the data of moisture content in both the species of fish, it was found to be higher in Green chromide. (Figure 6)

Ash content

In Green chromide, since $p>0.05$, it does not show any significant variation in the ash content across the months.

In Grey mullet, since $p>0.05$, there is no clear difference in the ash content across the different months studied.

Highest levels of ash content in Green chromide were observed in the month of October whereas for Grey mullet, highest levels were observed in the month of July. The ash content levels in Green chromide and Grey mullet were found to drop in the November and August respectively. (Figure 7)

Vitamin C

In Green chromide, since $p>0.05$, no significant variation in the vitamin C levels across the months is observed.

In Grey mullet, since $p>0.05$, there is no clear distinction in the vitamin C levels across the months studied.

Out of all, the biomolecules studied, the levels of vitamin C were the lowest. Both the fish species showed a similar trend in the level of vitamin C throughout the course of months for which it was tested. Green chromide showed the highest level of vitamin C in the month of December and for Grey mullet, the highest level was found to occur in the month of October. Low levels of vitamin C were observed for both the species of fish in the month of January. (Figure 8)

Sodium

In Green chromide, since $p>0.05$, No significant variation in the sodium levels across the months studied is seen.

In Grey mullet, since $p>0.05$, there is no significant difference in the sodium levels across the months studied.

. Highest content of sodium was observed in Green chromide in the month of December where in there was a sudden spike in the levels of sodium. However, along the other months, the sodium levels were similar in both the species of fish tested. (Figure 8)

Potassium

In Green chromide, since $p>0.05$, it does not show any significant variation in the potassium levels across the months studied.

In Grey mullet, since $p>0.05$, there is no clear distinction in the potassium levels across the months studied.

A significant variation in the levels of potassium was observed in both species of fish studied. The highest potassium content was observed in Green chromide in the month of December whereas the lowest was observed in the month of August. For Grey mullet, highest levels were observed in June and lowest were found to occur in January. (Figure 9)

Iron

In Green chromide, since $p>0.05$, it does not show any clear variation in the iron levels across the months studied.

In Grey mullet, since $p>0.05$, there is no significant difference in the iron levels across the study period.

The levels of iron in Green chromide were initially found to be almost similar in June and July and later decreased in the month of August. However, in Grey mullet the opposite was observed wherein the levels of iron were initially low in June and July but increased in the month of August. (Figure 10)

Zinc

In Green chromide, since $p>0.05$, it does not show any significant variation in the zinc levels across the period of study.

In Grey mullet, since $p>0.05$, there is no clear difference in the zinc levels across the months studied.

In both the species of fish a similar trend in the levels of zinc was found to be observed. The levels were found to be same in both the fishes in the month of August. In June and July higher levels were observed in Green chromide than Grey mullet. (Figure 11)

Fatty acids

Fatty acids present in the oil extracts of both the species of fish were analysed by using GC-MS. A higher number of fatty acids were found to be observed in the extracted oil samples of Grey mullet as compared to Green chromide. (Figure 12 and 13)

DISCUSSION

DISCUSSION

The nutritional profiling of the fishes is carried out to fulfil many purposes such as economic use, pharmaceuticals and for carrying out research. The health of an organism is affected by levels of nutrients present. The nutrients also play a role in providing energy for carrying out the metabolic activities and also affect the reproduction. The levels of the nutrients, that is the proteins, carbohydrates, amino acids and cholesterol is very crucial during the breeding season (Mule et al., 2017). The levels of these nutrients are seen to vary throughout seasons. In this study, several tests have been carried out to know about the levels of different biomolecules and their seasonal comparison in the 2 brackish water fish species, which is Green chromide (*Etroplus suratensis*) and Grey mullet (*Mugil cephalus*).

The action by which an organism mates or reproduces resulting in the production of an offspring is referred to as breeding. Each and every species of fish has a particular breeding season during which it reproduces. In addition to that, the breeding season consists of different phases, which is immature phase, pre spawning phase, spawning and post spawning phase. For Green chromide, the breeding in brackish water is throughout the year and the spawning season shows 2 peak periods which is May-July and November-February. The female moves from side to side and attaches the eggs one by one with the help of ovipositor on the surface of the nest. In the state fish of Goa breeding is seen to occur at various times in a year and the spawning is from October to January.

In the study of nutritional profiling of Green chromide and Grey mullet, a decline in the protein levels was observed in the months of October, November

and December and then it was found to again increase in the month of January. This trend was seen in both the species of fish studied. The decrease in the levels of protein during the months of October to December could be because since this period is the spawning period in both the fishes, most of the proteins are concentrated in the oocyte maturation and in providing energy for the gamete release. It could also be due to the certain environmental factors as well. The increase in the protein levels, indicates the preparation of fish for the breeding season. (Mule *et al*., 2017)

The carbohydrates are essential biomolecules which help in providing energy to carry out the metabolic activities but its part in influencing the reproduction in fishes is still not known (Mule *et al*., 2017). The carbohydrate concentrations for Grey mullet show an increase from September onwards whereas in Green chromide, it shows a decrease in the concentration values from October onwards. According to Mule *et al*., 2017, carbohydrates are not known to play any part in the reproduction of the fishes but they help in providing energy for other activities. The increase and decrease observed is due to the differences in the carbohydrate metabolism of the individual fish species which varies according to the season and ecological conditions where the fish species are found (Rudyk-Leuska *et al*., 2022).

Cholesterol acts as a precursor for the production of the steroid hormones which play a role in the spawning in fishes. In both the species of fish studied, the levels of cholesterol showed a decrease during the spawning season of both the species of fish. Initially in the months of June, July and August, the values almost remained the same in both the species of fish but later on showed a decreasing trend. Both Green chromide and Grey mullet are known to feed on

algae and detritus which contains high levels of cholesterol and hence this increase-decrease trend in the values is because of the feeding habit of the fishes. Also in addition it also depends on the quantity of algae each fish takes up and also the cholesterol levels are that present in various algal types (Norambuena *et al*., 2015).

The amino acid content in both the species of fish studied had almost similar levels across the period of months studied. In June, the concentration of amino acids was found to be highest since during this season, both the fishes are in their growing phase and require more amounts of amino acids for their development. While in the later months, the concentration of amino acids was found to decrease since this time is around the spawning season of both the fishes and most of the amino acids are concentrated in the ova that are formed (Mule et al., 2017).

According to Bezbaruah *et al*., 2021, the moisture content of the fishes depends on the temperature of the water body in which the fishes exist. Thus, the variation in the moisture content observed in Green chromide and Grey mullet is due to the differences in the temperature of the water body across different seasons.

The increase-decrease in the ash content of both the species of fish can be variable and depends on the mineral content in the muscle tissue of the fishes. Basically, high ash content in the fishes indicates an increased content of minerals like magnesium, calcium, potassium and zinc (Ayanda I. 2019).

Green chromide and Grey mullet have similar levels of vitamin C content. In both the fishes, the vitamin C is higher during the spawning periods as it is

required during this time to act as substrates or cofactors for the many metabolic reactions of the fish. In both the fishes it is observed that the vitamin C content is lower than the proximate composition. This is because even though the vitamin C is required by the fishes, the quantity required is very less (Mule *et al*., 2017).

Minerals such as sodium and potassium were analysed in both the species of fish using the flame photometer. Basically, these minerals are present in the water bodies where the fishes exist and hence the variation in the values is because of the difference in the uptake of these ions by individual fishes and also their levels present in each type of water body. Lower amount of these minerals suggests that the geographical area from which the fishes are collected which is brackish water contains low levels of sodium and potassium as compared to marine waters which have a very high concentration. Although lower in amount as compared to the other biomolecules these minerals play a very important role in maintaining an osmotic equilibrium between the cells and the extracellular medium (Presas-Basalo, 2022).

The iron and zinc levels in both the species of fish were found to be lowest of all the biomolecules and minerals tested since both these are minerals are trace minerals and required in low amount for growth and various metabolic activities. Hence the levels of these elements will depend again on the uptake from the water by individual fish species (Akram *et al*., 2019).

A wide variety of fatty acids were observed in the oil samples extracted from both the species of fish. A high number of omega 3 fatty acids were found to

occur in both the species of fish but more types were present in Grey mullet because of the high oil content present in it meat (Sengor *et al*., 2003)

CONCLUSION

CONCLUSION

In this study, analysis of the bioactive compounds and their seasonal comparison was carried out in the brackish water fishes and the protein, carbohydrate, fatty acids, cholesterol, amino-acid, vitamin and mineral content was determined and compared across the different months. The procurement of the fishes was done from the sluice gate located at Neugi-nagar. The study period was from June 2022 to January 2023. The tests for the estimation of different nutrients were carried out as given in the standard protocol. An extensive amount of variation was found in the nutrient content of the both the fishes through the study period. A significant variation was also observed in the ash and moisture content of the fish. Less amount of variation was observed in the vitamin C content of the fishes. Important omega 3 fatty acids were also observed to be found in both the species of fish whose presence was analysed by means of GC-MS. The mineral content was also analysed in both the species of fish. Although the mineral levels were low, these too play an important role in the metabolism, growth and osmotic maintenance of the fishes. Hence this study is very important as it helps us to understand the nutritive value and variation of nutrients in Green chromide and Grey mullet.

The data analysed in this study showed that both the species of fish have good protein, carbohydrate and fat value and hence both the fishes can be a healthy source of food if included in the diet. Mineral content although lower in amount can still prove to be beneficial since the dietary requirements are low. In addition to this, further studies can be done by taking into account the factors like climatic conditions, gender etc. of the fishes which can have impact on their nutritional value.

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