POPULATION STRUCTURE AND FEEDING ECOLOGY OF ENDEMIC GUDGEON *GOBIO INSUYANUS* LADIGES, 1960

ENDEMİK İNSUYU DEREKAYASI *GOBIO INSUYANUS* LADIGES, 1960 TÜRÜNÜN POPÜLASYON YAPISI VE BESLENME EKOLOJİSİ

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ABSTRACT

POPULATION STRUCTURE AND FEEDING ECOLOGY OF ENDEMIC GUDGEON *GOBIO INSUYANUS* LADIGES, 1960

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This study focuses on *Gobio insuyanus*, a local endemic and endangered fish species native to Turkish freshwaters. Of the 12 *Gobio* species found in Turkey, 11 are considered endemic to the country. *Gobio insuyanus* occurs in a few small springs in the Insuyu stream.

Individuals collected from the Stream and Spring habitats varied between 16.6 mm and 145.4 mm. The maximum age of the species was determined to be VII, and it was found that I+ age individuals were dominant in the population. In terms of sex ratios, females were found to be dominant in the population (D:E; 1.64:1.00), while the sex of 47 individuals (15.6%) could not be determined. According to the data obtained from 198 fishes whose stomach was analysed, it was found that *G. insuyanus* is a omnivore that feeds mainly on the adult and larval stages of insects, amphipods and on zooplankton. Seasonal, ontogenetic and habitat (Spring and Stream) differences in the diet composition of the species were observed.

While Diptera larvae were the dominant food category in the Stream habitat, Gammaridae individuals were more common in the Spring habitat. Gammarid larvae were dominant in autumn, whereas odonate larvae became dominant in spring. The relative importance of dipteran larvae in the diet was highest in summer. Taking into account the ontogenetic differences in feeding, it was found that adults showed a slightly higher specialisation and therefore a lower diet diversity. The adults fed mainly on gammarids, whereas the juveniles, which had a higher diet diversity, fed mainly on dipteran larvae. There was no significant difference in diet between the sexes. It was found that the trophic level of the species varied between 3.25 and 3.35 when unidentified detritus was excluded from the diet.

Keywords: *Gobio insuyanus*, Population Structure, Feeding Ecology, Endemic Gudgeon

ÖZET

ENDEMİK İNSUYU DEREKAYASI *GOBIO INSUYANUS* LADIGES, 1960 TÜRÜNÜN POPÜLASYON YAPISI VE BESLENME EKOLOJİSİ

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Bu çalışma, Türkiye tatlı sularına özgü, nokta endemiği ve tehlike altındaki bir balık türü olan *Gobio insuyanus*'u ele almaktadır. Türkiye'de bulunan 12 *Gobio* türünden 11'i Türkiye'ye endemiktir. *Gobio insuyanus*, tarımsal ve evsel amaçlı aşırı su kullanımı, azalan yağışların yol açtığı kuraklık ve tarımsal kirlilik gibi tehditerin ön planda olduğu Konya Kapalı Havzası'nda İnsuyu vadisinde bulunan birkaç küçük kaynakta yaşamaktadır. Bu açıdan ele alındığında *G. insuyanus* Dünya Doğayı Koruma Birliği (IUCN) kırmızı listesinde kritik düzeyde tehlike altında (CR) kategorisinde sınıflandırılmış ve acil koruma önerilmiştir. Söz konusu tez çalışmasında kullanılan balık örnekleri, Haziran 2018 ve Eylül 2019 ayları arasında Insuyu deresi ve bu dereyi besleyen en büyük kaynaktan elde edilmiştir. Bu çalışmada, türün boy, ağırlık, yaş ve eşey dağılımını kapsayan populasyon yapısı ve türün beslenme ekolojisi incelenmiştir. Dere ve kaynak habitatlarından örneklenen toplam 301 bireyin çatal boyu 16.6 mm ile 145.4 mm arasında değişim göstermiştir. Türün maksimum yaşı VII olarak belirlenmiş, I+ yaşındaki bireylerin populasyonda sayıca baskın olduğu ortaya konmuştur. Eşey dağılımı bakımından incelenen populasyonda dişilerin baskın olduğu gözlenirken (D:E; 1,64:1,00), 47 bireyin (%15.6) eşeyi belirlenememiştir.

Sindirim kanalı incelenen 198 balıktan elde edilen verilere göre *G. insuyanus* türünün böcek ergin ve larvaları, Amphipod ve Zooplankton üzerinden ağırlıklı beslenen bir karnivor olduğu belirlenmiştir. Türün besin kompozisyonunun habitatlar arasında (kaynak ve dere) olduğu gibi, mevsimsel ve ontogenetik olarak da farklılık gösterdiği ortaya konmuştur. Dere habitatında Diptera larvaları baskın besin kategorisi iken kaynak habitatında Gammaridae bireyleri öne çıkmıştır. Gammaridler yine genel olarak sonbahar mevsiminde baskın iken bahar aylarında Odonat larvaları baskın hale gelmiştir. Yaz aylarında ise Diptera larvalarının besin kompozisyonundaki göreli önemi en yükek olarak belirlenmiştir. Beslenmedeki ontogenetik farklılıklar ele alındığında ergin bireylerin biraz daha özelleşme gösterdiği, dolayısıyla daha besin çeşitliliğinin daha düşük olduğu ortaya konmuştur. Erginler ağırlıklı olarak Gammaridler üzerinden beslenirken, besin çeşitliliği daha yüksek olan yavru bireylerin daha çok Diptera larvası üzerinden beslendiği belirlenmiştir. Beslenme açısından eşeyler arasında anlamlı bir farklılık gözlenmemiştir. Besin içeriklerinden tanımlanamayan detritus (döküntü/kalıntı) kategorisi hariç tutulduğunda türün trofik düzeyinin 3.25 ile 3.35 arasında değiştiği belirlenmiştir.

Anahtar Kelimeler: *Gobio insuyanus*, Popülasyon Yapısı, Beslenme Ekolojisi, Endemik Taşkayası

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SYMBOLS AND ABBREVIATIONS

Symbols

Σ	Summation
a	Regression coefficient
b	Regression coefficient
DC *ij*	Fraction of *j* in the diet of *i*
D	Simpson's index
Df	Degrees of freedom
F	Female
G	Total number of stomachs containing food items
g	Guts containing prey
H	Shannon's Diversity Index
M	Male
MS	Mean Squares
N	Total number of individuals in the gut
n*i*	Number of individuals in the *i*th species
%N	Percentage of organisms in a specific food category
%FO	Frequency of Occurrence
%PSA	Prey – Specific Abundance
%V	Volume of organisms in a specific food category
P*i*	Number of individuals belonging to the *i*th species in each sample
T	Total length of fish
W	Weight of fish
x	Known length
Y	Length estimated

Abbreviations

Au	Autumn
AZE	Alliance for Zero Extinction
B	Levin's Niche Breath Index
CAP	Canonical Analysis of Principal Coordinates
CR	Critically Endangered Species
FI	Fullness Index
FL	Fin Length
HA	Habitat
IUCN	International Union for Conservation of Nature
Immat.	Immature
KBA	Key Biodiversity Area
LWR	Length –Weight Relationship
Ma	Maturity
Mat.	Mature
NaOH	Sodium hydroxide
NMDS	Non-metric Multidimensional Scaling
NORMSEP	Separation of the Normally distributed components of size – frequency samples
PAST	Paleontological Statistics Software Package
(P(perm))	Permutation – derived *p* - values
PERMANOVA	Permutational Multivariate Analysis of Variance
RGL	Relative Growth in Length
Sea	Season
SE	Standard Error

SL	Standard Length
Sp	Spring
SS	Sum of Squares
Su	Summer
TL	Total Length
TROPH	Fractional trophic level
TROPH*j*	Fractional trophic level of prey(*j*)
Ts*i*	Total volume of all stomachs, which contains the prey category "*i*"
Wi	Winter

1. INTRODUCTION

Freshwater ecosystems are a critical focus for global biodiversity, even though they cover only a fraction of the Earth's surface - less than 1%. Remarkably, these ecosystems function as the natural habitat for approximately 25% of all vertebrate species [1]. This figure is a potent reminder of the essential ecological role played by freshwater ecosystems, rather than a mere numerical interest. These environments serve as not only a breeding ground for vertebrate life, but also support a diverse range of invertebrate species, plants and microbial communities, forming complex and interdependent ecological networks. Moreover, the dynamic nature of freshwater habitats—ranging from rivers and lakes to wetlands and estuaries—provides a multitude of ecological niches, thus facilitating high levels of species richness and endemism. Nevertheless, the resilience of these ecosystems remains balanced at a critical threshold. Research has shown that there is a higher rate of biodiversity decline in freshwater habitats compared to that in terrestrial ecosystems [2, 3]. This alarming trend necessitates a closer look at the various factors contributing to the fragility of these vital ecosystems. Excessive water use for agricultural and industrial pursuits is reducing the availability of high-quality habitats. Pollution, such as nutrient overload and chemical pollutants, is altering water quality, thereby affecting species survival and reproduction. Modifications to natural water flow patterns, including dam construction and water diversions, disrupt the natural life cycles of numerous species. Degradation of riparian zones and wetlands further compounds these challenges. Additionally, the introduction of non-native species can outcompete or prey upon native species, disrupting established ecological relationships. The need for conservation is further compounded by the emerging threat of climate change, which introduces an additional layer of complexity. Changing precipitation patterns and increasing temperatures can result in altered water availability and quality, adding stress to already vulnerable ecosystems. This is particularly concerning in semi-arid and arid regions, where freshwater sources are naturally limited and further jeopardized by human-induced changes. In this global overview of pressing environmental challenges, specific regions - each with its own unique biodiversity and conservation issues - can serve as illustrative case studies of the wider complexities facing freshwater ecosystems.

Turkey, for example, represents a significant confluence of biodiversity due to its unique geographical position bridging two major continents - Europe and Asia. Further contributing to this richness are diverse climatic regions and the presence of three globally recognized biodiversity hotspots: the Caucasus, Irano-Anatolian, and Mediterranean. The convergence of distinctive geographic and climatic conditions has created an exceptionally rich and diverse environment for a wide range of freshwater fish species. Current scientific literature has identified over 375 fish species inhabiting inland waters of Turkey, representing 20 taxonomic orders and 34 families. Notably, more than half of these fish species are endemic to Turkey [4]. In addition to the factors mentioned above that contribute to the richness of biodiversity, geological events in the past have also had a significant impact on the formation of isolated populations, which in turn have facilitated the evolution of endemic species [5]. However, this high biodiversity exists in a landscape with significant conservation barriers. Turkey ranks 140th out of 163 countries in biodiversity and habitat conservation, a ranking that highlight the escalating threats it faces, particularly in the last decade [6]. Challenges include uncontrolled urbanisation, damming, draining wetlands, poaching and excessive irrigation. The current trend for development projects, particularly in water use, is intensifying these threats, leading to habitat loss and degradation, particularly in Central Anatolia. It is evident that centuries of human activity, especially since the middle of the 20th century, have had a significant impact on the native ecosystems of Turkey, leading to an urgent need for their conservation.

Within the broader context of global and regional freshwater biodiversity, the Insuyu karst sub-basin is an example of the irreplaceable and uniqueness of both its biodiversity and its vulnerability. Located within the wider closed Lake Tuz Basin, this sub-basin has been identified as a Key Biodiversity Area (KBA). In addition, the site has been proposed as an Alliance for Zero Extinction (AZE) site due to its globally significant importance as the only known habitat of the critically endangered fish species *Gobio insuyanus* (Cihanbeyli Gudgeon) and five other restricted fish species threatened by ongoing threats in the area [7]. Severe drought, amplified by agricultural water use, is one of the main threats. This water abstraction, along with regulations affecting water levels, poses a direct threat to the survival of *Gobio insuyanus* by eliminating its natural habitat. Additional challenges are posed by pollution, which is inferred to have originated from intensive agriculture, further threatening the fragile ecosystem and the species it supports. In this context, the Insuyu karst sub-basin serves as a localised example of the wider challenges facing freshwater ecosystems worldwide. Its ecological importance, as evidenced by its KBA and proposed AZE statuses, contrasts sharply with its vulnerability, making it a central focus for both local and global conservation efforts. In this sense of unique vulnerability, it is clear that specialised studies with a focus on endemic species are not only relevant, but crucial. An in-depth understanding of the biology of endangered species is essential to develop effective conservation strategies. Even in geographical regions where information has been collected for centuries, many new fish species are scientifically described and named every year [8].

However, there is not the same amount of information about the life history of each fish species. In fish, most studies on life history traits (age, growth, reproduction), dietary spectrum and feeding strategy have generally been conducted on economically valuable fish. There is however a serious lack of the most basic biological data on many species: this is a particular problem for small-sized species [8]. In particular, the study of feeding ecology is crucial; it not only elucidates an organism's role in the food web, but also provides insights into its trophic niche and resource use within the ecosystem. These aspects, in turn, are essential for evaluating the species' vulnerability to changes in food availability and habitat quality, factors that are often overlooked yet crucial for survival.

Consequently, this thesis aims to fill a critical gap in our knowledge by providing the first comprehensive analysis of both the population structure and feeding ecology of the critically endangered *Gobio insuyanus*. By studying its feeding habits, this study will shed light on the ecological role and resource use of the species and its potential interactions with other species, providing essential data for the design of targeted conservation measures. This detailed insight is not just an academic exercise, but a conservation necessity. It provides us with the tools to develop strategies that not only protect *Gobio insuyanus*, but also create a cascade of benefits for other species that share its increasingly fragile habitat.

2. PREVIOUS STUDIES

2.1 Biological studies on *Gobio* and *Gobio insuyanus*

The sole investigation pertaining to the biological characteristics of this species has been the length-weight relationship of the species. [9, 10, 11]. The first study was performed by

Erkakan *et al.*, was a study on the length–weight relationships for five Cyprinid species in Turkey, including two *Gobio* species [9]. In Insuyu Stream, Cihanbeyli- Konya, the species *Gobio insuyanus* was studied with a sample size of 18 specimens. According to this study, the fork length ranged from 6.9 to 13.6 cm, while weight varied between 3.9 and 28.6 g. The parameter '*b*' of the LWR had a value of 3.1.

Ergönül *et al.* examined a total of 53 individuals of *G. insuyanus* [10] and recorded a fork length range varying between 4.4 cm and 16.1 cm, and weight from 0.9 g to 57.2 g. From the regression analysis, the parameters '*a*' and '*b*' were determined as 0.01 and 2.9, respectively.

In another study by Şenyiğit and Mazlum, 468 individuals from 13 *Gobio* species were examined. Length-weight and length-length relationships of these species were analysed and the *b* value of 8 specimens of *G.insuyanus* was reported as 2.7, where the minimum and maximum values were respectively 2.3 and 3.1 [11].

Although there are no studies on the life history of *G. insuyanus*, there are studies on the growth and reproduction characteristics of *Gobio gymnostethus* by Özdemir [12]. In her research on the growth and reproduction biology of *G. gymnostethus* living in Melendiz Stream, it was found that age V was the dominant group and age 0 and age VII were the least represented. The fork length of *G. gymnosthethus* ranged between 30 to 151 mm.

Özdemir and Erk'akan worked on the growth and reproductive properties of the endemic species *Gobio hettitorum*, in Yeşildere Stream, Karaman, Turkey [13]. They examined 498 specimens, their fork lengths spanned between 30 mm and 161 mm. Males, which constituted 44.97% (224 specimens), had fork lengths ranging from 38 mm to 150 mm and weighed between 0.5 g and 35.3 g. Females made up 47.99% (239 specimens) of the population, with fork lengths from 55 mm to 161 mm and weights from 1.7 g to 64.1 g.

The study of *Gobio bulgaricus* Drensky, 1926 in the Istranca Stream provides insights into their growth patterns across different age classes [14] . Starting from juveniles in age class 0 with standard lengths of 2.4 - 3.3 cm and weights from 0.254 - 0.710 g, the fish grow to reach lengths of 7.5 - 9.5 cm and weights of up to 20.846 g in age class V. The diet of *G. bulgaricus* in the Istranca Stream predominantly consists of insects, particularly from the Diptera order, accounting for a 96.49% index of relative importance. While insects, in general, have an 83.96% frequency of occurrence, they make up 67.79% of the diet by weight and 91.77%

numerically. Other notable components of their diet include plant materials, crustaceans, and detritus. Some less common dietary items are annelids, arachnids, and bivalves [14] .

In the Karasu Stream, a total of 90 *G. bulgaricus* individuals were examined [15]. The age distribution is spread across four age groups between I to IV. When considering the entire population, females make up 37.78%, males account for 58.89%, and juveniles represent 3.33%.

In Turkey. studies regarding the biology of *Gobio* species are very few, so studies about *G. gobio* may give some idea about age and length distribution of *Gobio* species.

Some aspects of the biology of gudgeon *Gobio gobio* (L.) in Irish waters were studied as *G. gobio* was locally important as a food for predatory fish [16]. Where gudgeon are numerous, as in parts of the Lee and Blackwater catchments, their role in the ecology of the stream or river may be underestimated. As part of a general programme of research on Irish coarse fishes, some data on the spawning, food and growth of gudgeon were obtained and are considered in this paper [16]. The food organisms present were usually much crushed and broken up and identification to species was not always possible. The post-abdomens of cladocera were often intact and could be relied on for identification in many instances, while the remains of carapaces made it possible to identify at least the genus in other cases. In running water, cladocera and copepods were of minor significance in gudgeon diets, with *Gammarus*, nymphs of Ephemeroptera, larvae of caddis and chironomids, mollusks, and filamentous algae being the primary invertebrate foods. In standing waters, such as the Lee Reservoirs, however, exhibited a greater importance of cladocera and copepods, especially in smaller gudgeon. Larger reservoir gudgeon primarily consumed chironomid larvae, other aquatic insects, mollusks, and some plant material. Detritus, likely residues from invertebrates, silt, and plant fragments, was present in many gudgeon samples [16].

Despite these severe threats, there have been no comprehensive conservation actions specifically aimed at *G. insuyanus* as of the current date. The species' survival is largely dependent on the protection of its only habitat, the Insuyu Stream, from environmental degradation and pollution. Therefore, intensified research and conservation efforts are urgently needed to safeguard the continued existence of this unique Anatolian species.

Gobio insuyanus was recognised as a critically endangered species (CR) [17]. Therefore, there is an urgent need to expand the biological information about *G. insuyanus* in order to carry out conservation studies to ensure the survival of the species. The aim of this study is to determine the population structure and feeding habits of *G. insuyanus* in terms of understanding its role in the ecosystem and to contribute to the understanding of the biology of this species.

2.1 Taxonomic studies on *Gobio insuyanus*

The *Gobio* genus (Subfamily: Gobioninae) has 12 species in Turkish freshwaters, 11 of which are endemic to Turkey [18].

Gobio insuyanus is known as "Cihanbeyli Derekayası" in Turkish and this species is referred to in English as the "Cihanbeyli gudgeon" [18]. It is restricted to a sub-basin (particularly Insuyu Stream) in the west of the wider Lake Tuz Basin, [18, 19]. The species is distinguished from other species of *Gobio* in Anatolia by having 39–45 total lateral-line scales, head length 25–30% SL, breast completely scaled, scales extending forward to or almost to isthmus, 8–10 scales between posterior extremity of pelvic base and anus, scales on belly approximately equal to pupil diameter, 8–9 scale rows between dorsal origin and lateral line, predorsal length 48–52% SL, (Fig. 2.1) . The pharyngeal teeth are arranged in two rows and exhibit diverse patterns. The body is elongated and moderately compressed laterally, gradually narrowing towards the caudal base. The mouth is terminal or slightly inferior, with thick lips, particularly the lower one. It has a single pair of maxillary barbels. The head is wide and slightly flattened at base [20]. It is a bottom dwelling, small species with a midlateral row of dark-brown or grey blotches on flank and spotted body and fins. This species inhabit springs and spring associated streams with slow to moderately fast-flowing waters, on sandy and gravel bottom, often amongst very dense aquatic vegetation. Figure 2.1 shows an image of an adult individual.

Figure 2.1. *Gobio insuyanus* from Insuyu Stream. Photograph courtesy of Dr. Baran Yoğurtçuoğlu

Gobio insuyanus is assessed as a critically endangered species (CR) based on its very restricted distribution range with several ongoing threats, particularly drought and habitat loss. Most of the studies directly related to *G. insuyanus* are systematic [18,19, 20, 21, 22,23].

3. SITE DESCRIPTION

The Insuyu Stream is a water system found in the Konya province, within the district of Cihanbeyli in Turkey. It forms an integral part of the Insuyu Valley, a dry valley system extending in the east-west direction (Figure 3.1- 3.3).

Geographically, the Insuyu originates near the Insuyu village, flowing into the western coast of Tuz Gölü (Salt Lake). Its path meanders through the valley, carving out an impressive landscape of limestone cliffs, especially towards the west.

Figure 2.4 depicts a satellite image of the Insuyu Stream, with the two sampling locations, Spring and Stream shown. The water depth is about 50 cm in the Stream, while it is about 30-150 cm in the Spring. The total area of the Insuyu Valley, which the Insuyu Stream traverses, is approximately 75.23 square kilometres [24]. Figure 2.2 and 2.3 depict general photographs of the Stream and Spring sampling locations, respectively.

Figure 3.1. Photograph of the Stream sampling location. Photograph courtesy of Dr. Baran Yoğurtçuoğlu

Figure 3.2. Photograph of the Spring sampling location. Photograph courtesy of Dr. Baran Yoğurtçuoğlu

Figure 3.3. Satellite image of the Insuyu Stream, with the Spring and Stream sampling locations pinned

3.1 Climate

The Stream's surrounding areas have a longitude of 32.82ºE and a latitude of 38.69ºN. These coordinates place the Stream within a semi-arid climate region characterised by warm summers and mild winters, typical of Central Anatolia [25].

3.2 Fish Species

Fish species identified in the study area are as follows:

Gobionidae:	*Gobio insuyanus,*
Leuciscidae:	*Pseudophoxinus crassus,*
	Squalius sp.,
Nemacheilidae:	*Oxynoemacheilus axylos,*
	Seminemacheileus ekmekciae,
Cobitidae:	*Cobitis turcica*
Aphaniidae:	*Anatolichthys anatoliae*

3.3. General Characteristics of the Area

Interestingly, the Insuyu Stream also serves as a significant ecological corridor. It hosts several endemic plant species such as *Achillea sieheana* and *Astragalus kırsehehiricus*, which add to the unique character of the region [26]. Human activities around the Insuyu Stream and Valley are predominantly characterised by agriculture. The valley, owing to its fertile soils, provides a suitable environment for growing a variety of crops. Agricultural activities are especially intensive in the Cihanbeyli region, which forms part of the valley. Crop farming contributes significantly to the local economy, while simultaneously shaping the landscape around the Stream. Livestock farming is another major human activity in the region. The presence of extensive steppe grasslands, particularly towards the eastern end of the valley, supports grazing for cattle, sheep, and goats. These livestock animals play a crucial role in the local livelihoods and contribute to the region's socio-economic dynamics [26]. Aquaculture is also present in the region, demonstrated by the establishment of a fish farm along the Stream. This enterprise further illustrates the importance of the Insuyu Stream as a natural resource. Aquaculture, if managed sustainably, can provide both food and economic stability for local communities. However, it also needs careful monitoring to avoid potential impacts on the native aquatic species, particularly the endemic and endangered ones. Agriculture, livestock farming, and aquaculture are the main human activities around the Insuyu Stream and Valley. While these activities support local economies and livelihoods, they may also pose potential environmental challenges, such as eutrophication, which could significantly impact the Stream's ecosystem and biodiversity [26].

In the context of the Insuyu Stream, which houses globally endangered fish species in addition to *Gobio insuyanus*, eutrophication could pose a significant threat. These species, already under pressure due to their limited distribution, could be further endangered if their habitat becomes hypoxic.

Given these potential impacts, it is crucial that farming and aquaculture practices in the Insuyu Valley are conducted sustainably. This includes using fertilizers judiciously in agriculture, properly managing livestock waste, and ensuring that aquaculture systems are designed to minimise nutrient release into the Stream [27].

The Cihanbeyli-Yunak highway that stretches along the valley is another significant mark of human influence in the area. This infrastructure plays a vital role in facilitating transportation and economic activities but can also potentially affect the Stream's habitat, particularly through the risk of habitat fragmentation [28].

Despite these anthropogenic activities, no specific threats to the Insuyu Stream and its habitats have been reported. Nonetheless, ongoing monitoring and management strategies are essential to ensure that human activities do not negatively impact the unique biodiversity and ecological health of the area.

4. MATERIAL & METHODS

4.1. Data Collection

Fish samples were obtained by using a hand-held dip-net with a 1 mm mesh size, in addition to a fyke net measuring 3 x 1.5 x 1.5 cm with a knot-to-knot 4 mm mesh size. The sampling for this study took place between June 2018 and September 2019, with the main collection sites being at the Spring and Stream locations. Regular monthly samplings of fish, within the stated period, were conducted.

4.2. Population Structure

After collecting the fish specimens in the field and transporting them to the laboratory, their lengths were measured using a Yamayo digital caliper, model IP54, with a sensitivity of 0.05mm. The standard, fork and total lengths (SL, FL and TL) of individuals were recorded. Fish weights were measured, using a Sartorius CPA623S digital balance with a sensitivity of 0.001 g after their lengths were recorded.

To facilitate the conversion between fork length (FL), standard length (SL), and total length (TL) in the sampled fish species, we employed linear regression models. The length-length relationships were modeled as linear functions, represented by the equation:

$Y = a + bX$,

Where Y is the length to be estimated, X is the known length, and a and b are regression coefficients. The coefficients a and b were estimated using least squares linear regression. A linear regression model to determine the relationship between total length and weight was used. The log transformed data was used to estimate the parameters of $W = a \times L^b$ equation. According to the following equation: $\log W = \log a + b \log L$; where W represents the total weight of the fish, L represents the total length of the fish, a indicates the intercept of the log-log relationship line and b is the regression coefficient.

Age was determined using fish scales, specifically extracted from the right flank of *G. insuyanus* specimens, situated between the base of the pectoral and dorsal fins [29,30]. Once extracted, these scales were prepared for examination by first immersing them in water for a couple of minutes, then by delicately scrubbing them in a 4% NaOH solution to eliminate mucus and pigments, followed by a rinse in water. Once cleaned, the intact and clean scales were immersed in 96% ethyl alcohol for 3 to 4 minutes to remove the water from them, and the clean scales were mounted between two microscope slides. The ready scales were then analysed under an Olympus SZ-X12 model stereomicroscope.

Besides the scale readings, the length frequency analysis method was also employed to assess age. The Modal Progression Analysis, a bi-stage technique that infers age from alterations in length frequency peak values, was used. To begin with, the size groups assumed to signify cohorts or age classes in the seasonal length frequency sampling were recognised, as per the methodology described by Bhattacharya [31]. Subsequently, the NORMSEP method was employed to distinguish the constituents of length-frequency samples that displayed a normal distribution. A separation index (SI) exceeding 2 was targeted to acquire significantly discrete component groups. The correlation between total length and weight was analysed individually for female and male specimens, as well as for the combined dataset [32].

4.3 Assessment of Feeding Habits

The evaluation of stomach contents of the sampled fish provided insights into their dietary habits. To identify the organisms from the stomach content, an Olympus SZ-X12 model stereomicroscope was used, and their volumes were calculated using a counting chamber (Sedgewick Rafter) [33]. The volume of the organisms was estimated either by compressing the food content into a calculable area, or by approximating the volume of the organism's closest geometric shape [34]. The organisms were identified using literature such as the following, [35, 36].

To determine the feeding intensity, the Fullness Index (FI) has been used; the equation

FI = (stomach content weight x 100) / fish body weight

was utilised in the calculation of the index [37, 38].

The frequency of occurrence (%FO) was calculated to establish the regularity with which particular organisms appeared within the dietary samples being examined. This index was computed based on the proportion of the stomachs that included a given food category relative to the total count of stomachs. A significant element of research into stomach content is identifying those organisms within a species' habitat that make the largest dietary contribution. For this, the frequently used equation is the IRI (index of relative importance).

The Index of Relative Importance (IRI), is calculated by: IRI = %FO x (%N + %V)

The equation was used to establish the relative importance of each food item, where %FO is the frequency of occurrence of the food category, %N is the number of organisms in a specific food category to the total number of food organisms, and %V is the volume of organisms in a specific food category to the total volume of food organisms [39].

$$\%N = n/N \times 100$$

$$\%V = v/V \times 100$$

$$\%FO = g/G \times 100$$

Where:

N = Number of Total Prey

n = Prey

V = Total Volume

v = Volume of prey

G = Total number of Guts containing the prey

g = Guts containing prey

4.4. Assessment of Feeding Ecology

One of the aims of this study was to investigate the feeding ecology of *G. insuyanus* in relation to a variety of environmental and biological factors. This was done by assessing dietary diversity, niche breadth, trophic level and feeding strategy of the species. The analysis was conducted by employing a dataset that considered the biomass of various prey items across factors such as seasons, habitats and maturity stages of the fish.

To estimate species diversity in the stomach contents, we employed the Shannon and Simpsons indices. The use of the Shannon index as a diversity index is justified as it considers both the number of individuals and the number of taxa. This index ranges from 0 for communities consisting of a single taxon to higher values for communities with numerous taxa, each with a small number of individuals. Unlike conventional methods that rely solely on species counts, our analysis was conducted by considering the biomass of each species in order to gain a better understanding of their ecological role. The Shannon Diversity Index was calculated according to the equation:

$H'=\sum[(p_i)\times\ln(p_i)]$,

where

p_i = number of individuals belonging to the i th species in each sample

and the Simpson's index (D), which emphasises the dominant group amongst food categories, were utilised.

$$D=N(N-1)\sum n_i(n_i-1)$$

Where;

n_i — Number of individuals in the i-th species; and

N — Total number of individuals in the gut.

We performed bootstrap resampling to generate 95% confidence intervals for the Shannon Diversity Index. The statistical analysis was executed using the Paleontological Statistics Software Package (PAST), version 3.20. Specifically, 9999 bootstrap replicates were conducted to estimate the confidence intervals for each calculated diversity index. For each bootstrap replicate, PAST randomly resampled the original biomass data with replacement, subsequently recalculating the Shannon Diversity Index for each resampled dataset. The 2.5th and 97.5th percentiles of the distribution of these bootstrapped indices were then used to define the 95% confidence intervals. The evenness with which individuals are distributed amongst the taxa present is regarded as the Shannon diversity divided by the logarithm of the number of taxa.

The primary metric used for niche estimation was the Levin's Niche Breadth Index (B), calculated as:

$$B = \frac{1}{\sum_{i=1}^{n} P_i^2}$$

Where pi is the proportion of the i^{th} prey item in the diet, and n is the total number of prey items. The proportion pi was calculated as the biomass of the *h*ith prey item divided by the total biomass for each factor. We used a bootstrapping approach with 1,000 iterations to calculate the confidence intervals for each factor's niche breadth index. In each iteration, we resampled the dataset with replacement and recalculated the niche breadth index. The 95% confidence intervals were determined by taking the 2.5th and 97.5th percentiles of the bootstrapped indices. Pearson correlation coefficients were calculated amongst the bootstrapped niche breadth indices to understand how they correlate across different factors. The analysis was carried out using the Python programming language, utilizing the Pandas package for the manipulation and calculation of data [40] while Matplotlib and Seaborn are employed for creating visual representations of data [41, 42].

The ACCESS stand-alone application TROPHLAB was used to assess the fractional trophic level (TROPH) and the associated standard error (SE) of *G. insuyanus* [43]. When available, the estimates were made for each studied case (e. g. sampling station, season, etc.). The

TROPH is computed based on the dietary constituents and the trophic levels of the prey items (as categorized int he following tables) based on the equation,

$$TROPH_i = 1+\sum_{j=1}^{G} DC_{ij} \text{ x } TROPH_j;$$

where $TROPH_j$ is the fractional trophic level of prey (j), DC_{ij} represents the fraction of j in the diet of i and G is the total number of prey species. TROPHLAB estimations are based on two different types of data: quantitative (volume or weight contribution of prey to the diet) and qualitative (frequency of occurrence of prey in the diet and other relative indices).

To investigate the feeding strategy of *Gobio insuyanus*, we utilised the modified Costello's graphical method [44]. This method plots the prey-specific volume of each food category against the frequency of occurrence (%FO) on a two-dimensional graph. In contrast to the traditional approach that relies on prey abundance data, we used prey volume data to account for uncountable food items. The prey-specific volume was calculated using the following formula:

$$Pi=(\sum Si/\sum Tsi) \text{ x } 100,$$

where Pi is the prey specific volume of “i” food category and “Tsi” is the total volume of all stomach, which contain prey category “i”.

To test the intraspecific (maturity stages and sexes) and seasonal and spatial differences in diet composition, permutational multivariate analysis of variance (PERMANOVA) was applied, using Bray-Curtis similarity resemblance matrices of square-root transformed volume data, with 9999 permutations. To assess ontogenetic variation in feeding fish we classified them into two maturity classes, "mature" and "immature". The categorisation process involved identifying a threshold of the length-at-first maturity, which we determined to be 41.3 mm fork length. Fish specimens below this value were considered immature, while those above were considered mature. Canonical Analysis of Principal Coordinates (CAP) was preferred over Non-Metric Multidimensional Scaling (NMDS) to visually represent the organisation of dietary patterns across different factors, including seasons, sexes, habitats and maturity classes. This preference was motivated by CAP's ability to perform constrained ordination, which allows for more targeted hypothesis testing and variance partitioning. It is important to note that, “detritus” was intentionally omitted in these analyses to enhance study precision. The rationale behind such exclusion is due to the uncertain compositional nature of detritus, which makes its impact on dietary patterns ambiguous. Furthermore, considering the predominance of detritus in the dietary data, it could potentially mask the true dietary preferences of the species studied, thereby introducing an inherent element of bias. By removing this category, our objective was to create a more precise and comprehensible representation of the dietary ecology of the species. SIMPER (Similarity Percentages) analysis was used to assess species contributions to within-group homogeneity and between-group heterogeneity in the Spring and Stream habitats. Bray-Curtis similarity indices were calculated to assess similarity, with a 90% cut-off for minor contributions. A one-way analysis approach was used, with each habitat type treated as a single factor with multiple replicates: 54 for 'Spring' and 56 for 'Stream'. The aim of the analysis was to identify species that significantly drove the similarities within and the dissimilarities between the habitat groups. PERMANOVA, SIMPER and MDS were applied by PERMANOVA+ v1.0.1. PRIMER v6 software package [45].

5. RESULTS

5.1. Population Structure

5.1.1. Length Distribution

The fork lengths (FL) of 301 *Gobio insuyanus* individuals sampled range from 16.61 mm to 145.44 mm. The smallest individual captured in terms of fork length was an immature individual measuring 16.61 mm. The gender of this individual could not be determined since it was immature. The largest individual, in terms of fork length, measured 145.44 mm and was a female. Amongst the individuals with identified genders, the smallest female measured 23.45 mm, and the smallest male measured 36.7 mm. The fork length distributions of *G. insuyanus* individuals are shown in Figure 5.1. When examining the clustering of fork lengths of individuals, all immature individuals (47) fall within the fork length range of 16.61-41.3 mm. For females, 50% (69) have fork lengths ranging from 23.45-38.09 mm. For males, 50% (37) have fork lengths ranging from 36.7-62.35 mm.

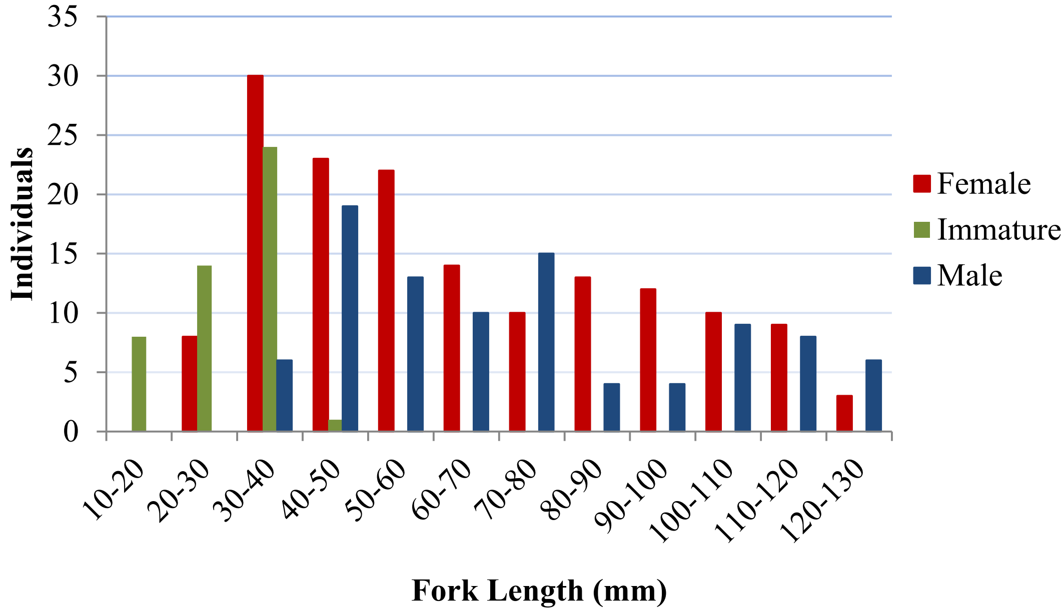


Figure 5.1. Fork Length Distributions of *G. insuyanus* individuals

5.1.2. Weight Distribution

The weights of *G. insuyanus* individuals in the dataset range from 0.049 grams to 54.221 grams. The smallest individual captured in terms of weight weighed 0.049 grams. This individual was immature, and its gender could not be determined due to its immature status. The largest individual in terms of weight weighed 54.221 grams, and it was identified as a female. Amongst the individuals with identified genders, the smallest female weighed 1.338 grams while the smallest male weighed 0.555 grams. The weight distributions of all sampled *G. insuyanus* individuals are shown in Figure 5.2.

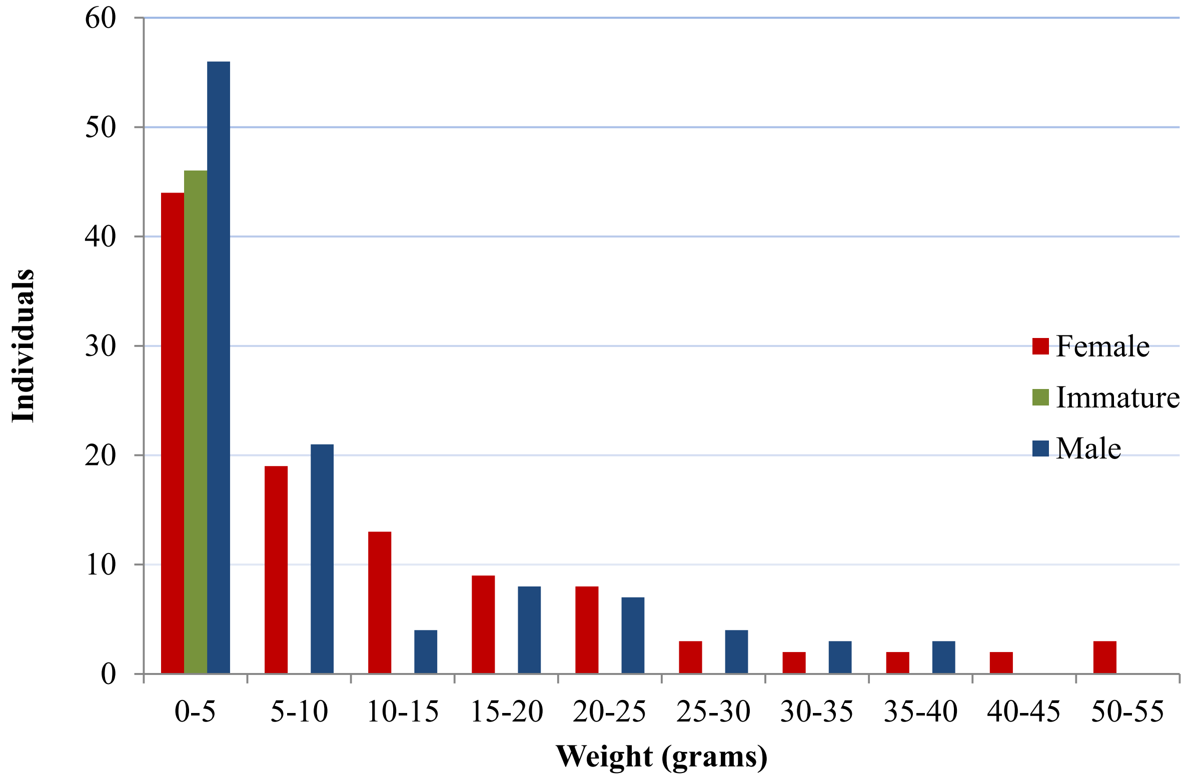


Figure 5.2. Weight distributions of *Gobio insuyanus* individuals

5.1.3. Age Distribution

Maximum age of *Gobio insuyanus* was found as VII. Age group 0, represented by 30 individuals. Age group I had the highest number of individuals with 64 specimens. Age group II had 39 individuals, and age groups III, IV, and V had smaller numbers, with 18, 19, and 11 individuals, respectively. These findings suggest that the population of *G.insuyanus* is composed of individuals primarily in the 0+, I+, and II+ age groups, with age group I+ being the most abundant (see Fig. 5.3).

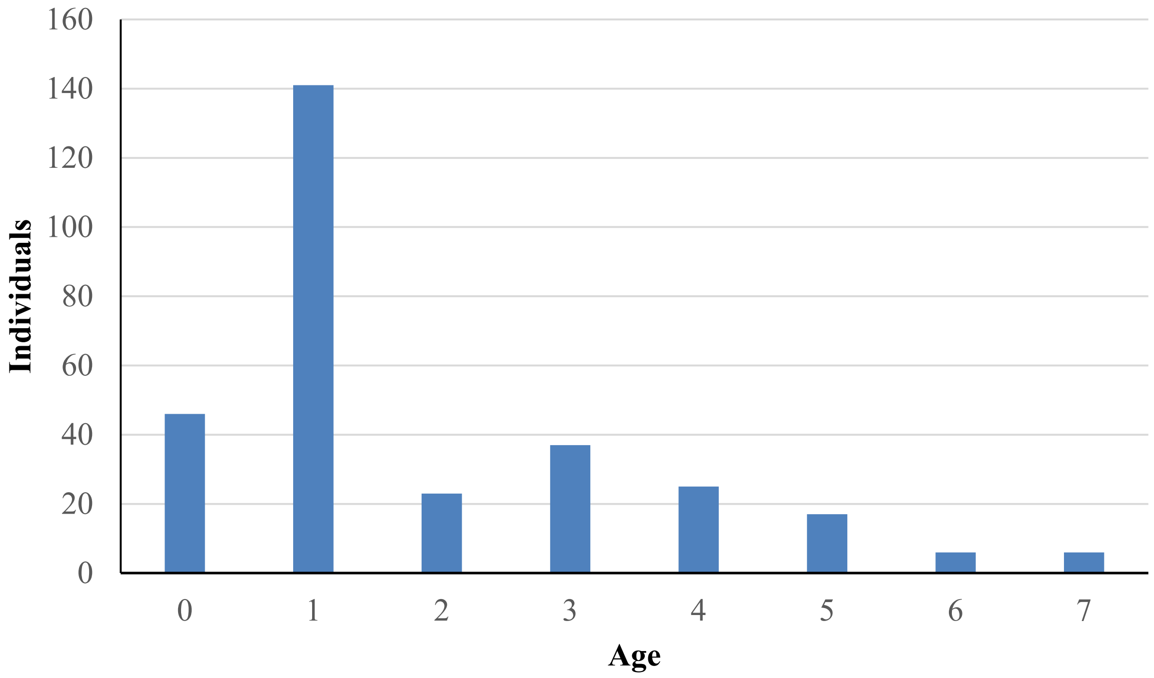


Figure 5.3. Age Distribution of *G. insuyanus*

5.1.4. Sex distribution

The sex distribution of *G. insuyanus* is characterised by a dominance of females, with 154 compared to 94 males, resulting in a female to male ratio of 1.64:1. Immature specimens comprise 47 individuals, whose sex is undetermined due to underdeveloped gonads. See Figure 5.4 for the sex distribution of the samples obtained from both Stream and Spring.

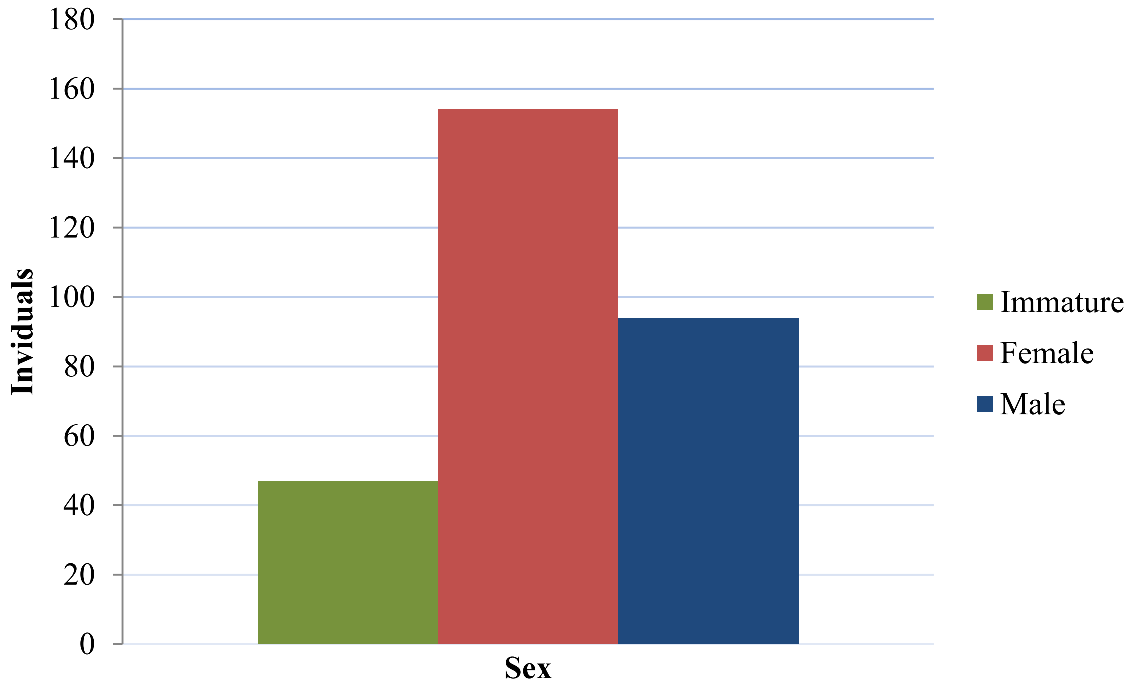


Figure 5.4. Sex Distribution of *G. insuyanus*

5.1.5. Length – Weight Relationship

The relationship between length and weight values of the Spring and Stream samples was compared using the *b* values, calculated through the Student's t-test (Table 5.1). According to the test results, it was found that the difference between the *b* values of the two groups was not statistically significant ($t = 1.769$, $p > 0.05$). Based on this result, the Spring and Stream values were combined for regression analysis. For the entire population, the length-weight relationship equation was determined as $W = 0.016\ FL^{3.27}$ (Figure 5.5)

Table 5.1. *G. insuyanus* Length – Weight Relationship Parameters

	n	a	SE (a)	*b*	SE (*b*)	r^2
Spring	164	-2,042	0,017	3,25*	0,021	0,993
Stream	136	-2,05	0,016	3,27*	0,022	0,994

**b* value difference from 3 (Student’s *t*-test t_{spring}= 11,9 p < 0,05, t_{stream} = 12,27 p<0,05).

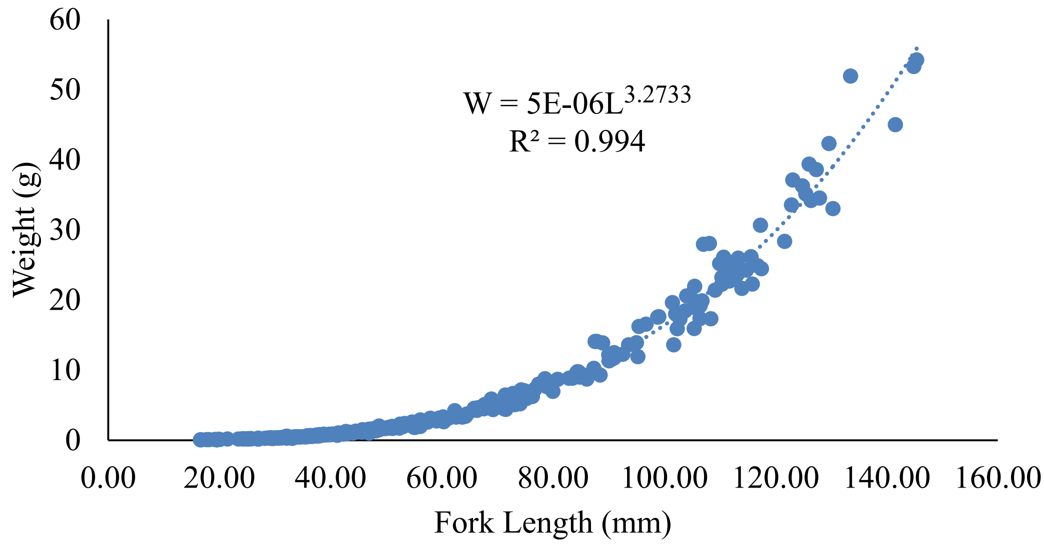


Figure 5.5. Length-Weight Relationship for *G. insuyanus*

5.2. Diet Composition and Feeding Activity

5.2.1. Fullness Index

The average fullness index values for a fish across different parameters, delineating seasonal and maturity variations are presented in Table 5.2. In the context of seasonal changes, the fish's fullness index is highest during the spring and summer months, with values of 26.21 and 28.08, respectively. Conversely, during the winter season, the fullness index drops to 19.88, indicating potential fluctuations in the fish's feeding behavior. The table also highlights distinctions between mature and immature fish, with mature individuals exhibiting a fullness index of 24.17 compared to 28.54 for immature ones. The fullness index for the Spring site was higher with an index of 26.21 when compared to the 22.44 for the Stream site.

Table 5.2. *G. insuyanus* average fullness index values across habitat, season; and ontogeny factors.

Parameter	Spring	Stream	Autumn	Winter	Spring	Summer	Mature	Immature
Fullness Index	26.21	22.44	23.40	19.88	22.33	28.08	24.17	28.54

5.2.2. Overall Diet Decription

In order to determine the diet composition of *G.insuyanus*, the stomach contents of a total of 198 individuals were examined, with 48 individuals from the Stream sampling location and 150 individuals from the Spring sampling location. Table 5.3 displays the index of relative importance (IRI) and its constituents (%N, %V and %FO) for Stream, Spring and Stream and Spring combined. Table 5.3 displays the IRI, %N, %V and %FO values for all seasons of Stream and Spring combined. Table 5.4 presents the IRI, %N, %V and %FO values for all seasons from the Stream habitat. Table 5.5 exhibits the IRI, %N, %V and %FO values for all seasons from the Spring habitat. The main food items identified in *G.insuyanus* individuals include adult Insecta (Corixidae, Curculionoidea, Diptera), Insecta larvae (Diptera, Odonata, Plecoptera), Zooplankton (Branchiopoda, Copepoda including their naupli, Ostracoda divided into benthic and planktonic types), other invertebrate groups (Amphipoda, Bivalvia Gastropoda, Acariformes, Nematoda), and vertebrates were only represented by fish larvae. Figure 5.6 displays a heatmap of proportions of prey types across factors using the volume of each prey category. The analysis revealed that the proportions of prey types exhibited significant variability across the studied factors. For instance, the Amphipoda showed pronounced abundance during the Spring and in Stream conditions, indicated by its more vibrant hue on the heatmap. Similarly, Gammaridae displayed elevated proportions in the same conditions. However, most prey types, including Ostracoda Suspended and Ostracoda Benthic types, consistently displayed medium to low proportions across the majority of factors. Additionally, a clear seasonal variation in prey abundance was observed, with certain prey types, such as Amphipoda, exhibiting higher proportions in specific seasons like Spring. When examining developmental stages, both the immature and mature categories predominantly displayed low proportions for most of the prey types. These findings suggest the presence of underlying ecological patterns or interactions influencing the abundance and distribution of these prey types across various conditions.

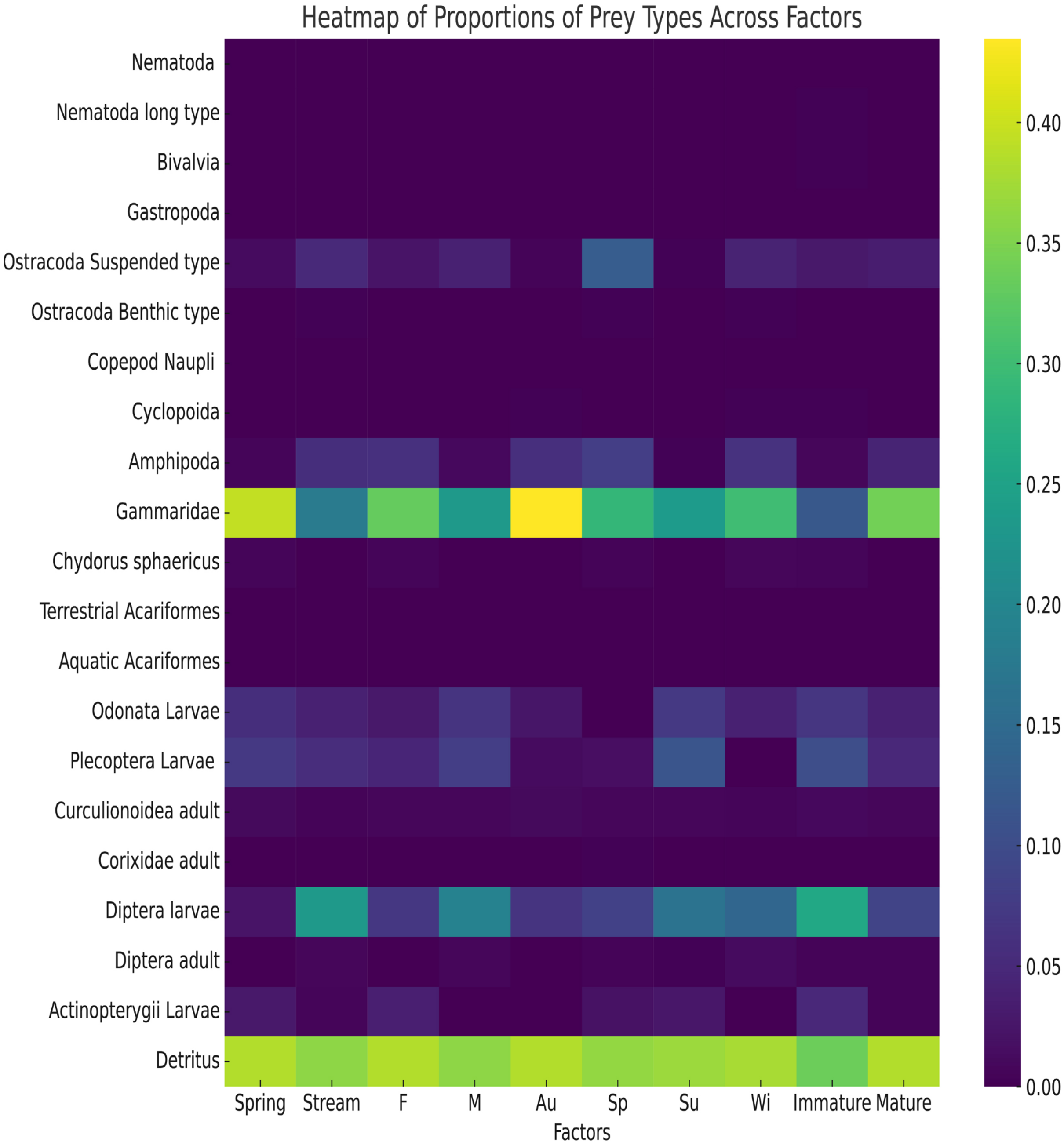


Figure 5.6. Heatmap of Proportions of Prey Items Across Factors

Table 5.3. N%, V%, FO% and IRI Values For Stream, Spring and Stream And Spring Combined.

Location >	**Stream**				**Spring**				**Stream + Spring**			
Prey item	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI
Animalia												
Insecta (adult)												
Diptera	2.526	0.763	15.385	50.595	0.636	0.109	6.557	4.883	1.900	0.457	11.111	26.190
Corixidae	0.079	0.055	1.538	0.206	0.159	0.063	1.639	0.364	0.106	0.059	1.587	0.261
Curculionoidea	0.158	0.466	3.077	1.920	0.636	1.064	6.557	11.145	0.317	0.745	4.762	5.057
Insecta (larva)												
Diptera	30.702	23.376	66.154	3577.489	5.246	2.262	22.951	172.319	22.269	13.512	45.238	1618.670
Odonata	0.631	3.974	9.231	42.513	1.590	5.666	11.475	83.262	0.950	4.764	10.317	58.958
Plecoptera	1.657	5.564	16.923	122.206	3.816	7.252	16.393	181.441	2.375	6.353	16.667	145.455
Zooplankton												
Branchiopoda												
Chydorus sphaericus	0.158	0.004	1.538	0.250	37.520	0.582	9.836	374.776	12.559	0.274	5.556	71.299
Copepoda												
Naupli	0.158	0.000	3.077	0.486	0.000	0.000	0.000	0.000	0.106	0.000	1.587	0.168
Cyclopoida	5.525	0.130	20.000	113.094	0.318	0.004	1.639	0.528	3.799	0.071	11.111	43.007
Ostracoda												
Ostracoda	41.674	5.135	26.154	898.369	17.806	1.262	36.066	615.364	33.773	3.325	30.952	930.71
Other invertebrates												
Amphipoda												
Amphipoda	2.289	5.709	10.769	86.126	0.477	0.674	3.279	3.772	1.689	3.356	7.143	36.036
Gammaridae	7.182	17.913	43.077	1081.030	27.981	39.514	59.016	3983.318	14.090	28.004	50.794	2138.115
Bivalvia												
Bivalvia	4.341	0.117	9.231	41.147	0.795	0.012	4.918	3.969	3.166	0.068	7.143	23.100
Gastropoda												
Gastropoda	0.158	0.044	3.077	0.622	0.636	0.101	6.557	4.831	0.317	0.071	4.762	1.844

Continuation of Table 5.3

Location >	Stream				Spring				Stream + Spring			
Prey item	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI
Acariformes												
Terrestrial type	0.000	0.000	0.000	0.000	0.477	0.036	1.639	0.841	0.158	0.017	0.794	0.139
Aquatic type	1.105	0.006	7.692	8.550	0.159	0.001	1.639	0.261	0.792	0.004	4.762	3.787
Nematoda												
Short type	1.421	0.000	4.615	6.557	0.000	0.000	0.000	0.000	0.950	0.000	2.381	2.262
long type	0.158	0.028	3.077	0.573	0.954	0.097	4.918	5.167	0.422	0.060	3.968	1.914
Vertebrates												
Fish larvae	0.079	0.630	1.538	1.091	0.636	2.874	3.279	11.507	0.264	1.678	2.381	4.624
Detritus	NA	36.086	86.154	NA	NA	38.428	88.525	NA	NA	37.180	87.302	NA

Table 5.4. N%, V%, FO% and IRI Values For All Seasons Of Stream And Spring Combined.

Season >	Autumn				Winter				Spring				Summer			
Prey item	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI
ANIMALIA																
Insecta (adult)																
Diptera	0.000	0.000	0.000	0.000	3.697	1.323	11.111	55.780	0.805	0.370	21.739	25.550	1.724	0.245	11.290	22.235
Corixidae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.161	0.171	4.348	1.445	0.172	0.057	1.613	0.370
Curculionoidea	0.787	1.144	4.348	8.398	0.176	0.616	5.556	4.400	0.161	0.724	4.348	3.847	0.517	0.719	4.839	5.983
Insecta (larva)																
Diptera	17.323	6.487	43.478	1035.230	15.669	14.130	33.333	993.305	7.085	8.208	43.478	664.948	46.034	16.500	50.000	3126.743
Odonata	0.787	2.438	4.348	14.022	0.528	3.937	16.667	74.427	0.000	0.000	0.000	0.000	2.414	7.152	14.516	138.863
Plecoptera	0.787	1.300	4.348	9.076	0.000	0.000	0.000	0.000	0.322	1.645	8.696	17.105	7.241	11.444	29.032	542.471
Zooplankton																
Branchiopoda																
Chydorus sphaericus	9.449	0.127	8.696	83.272	25.704	0.835	5.556	147.438	11.594	0.484	8.696	105.025	1.379	0.018	3.226	4.507
Copepoda																
Naupli	0.787	0.000	4.348	3.424	0.176	0.000	5.556	0.978	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cyclopoida	19.685	0.228	26.087	519.458	7.570	0.211	27.778	216.142	0.000	0.000	0.000	0.000	0.690	0.008	4.839	3.374
Ostracoda																
Ostracoda	8.661	0.519	30.435	221.695	31.162	4.498	66.667	1,680.129	69.083	13.052	47.826	3,120.4	3.966	0.235	14.516	60.977
Other invertebrates																
Amphipoda																
Amphipoda	4.724	5.796	13.043	137.220	2.113	6.241	16.667	139.226	2.093	7.944	8.696	87.285	0.172414	0.202438	1.612903	0.604599
Gammaridae	35.433	43.468	56.522	4459.649	10.211	30.164	83.333	3364.629	7.568	28.722	47.826	1735.634	20.172	23.685	40.323	1768.452
Bivalvia																
Bivalvia	0.787	0.010	4.348	3.469	2.641	0.084	16.667	45.415	0.000	0.000	0.000	0.000	7.586	0.096	8.065	61.953
Gastropoda	0.000	0.000	0.000	0.000	0.352	0.117	11.111	5.209	0.322	0.137	8.696	3.993	0.345	0.045	3.226	1.259

Continuation of Table 5.4.

Season >	**Autumn**				**Winter**				**Spring**				**Summer**			
Prey item	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI
Animalia																
Acariformes																
Terrestrial type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.483	0.099	4.348	2.530	0.000	0.000	0.000	0.000
Aquatic type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.161	0.001	4.348	0.706	2.414	0.007	8.065	19.520
Nematoda																
Short type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.103	0.000	4.839	15.017
long type	0.787	0.069	4.348	3.725	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.207	0.102	6.452	8.443
Vertebrates																
Fish larvae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.161	1.956	4.348	9.203	0.690	2.591	3.226	10.583
Detritus	NA	38.412	91.304	NA	NA	37.844	100.000	NA	NA	36.486	86.957	NA	NA	36.893	82.258	NA

Table 5.5. N%, V%, FO% and IRI Values For All Seasons From The Stream Habitat.

Season >	Autumn				Winter				Spring				Summer			
Prey item	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI
Animalia																
Insecta (adult)																
Diptera	0.000	0.000	0.000	0.000	5.497	1.779	20.000	145.523	0.442	0.178	18.182	11.281	2.507	0.558	22.222	68.120
Corixidae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.221	0.206	9.091	3.884	0.000	0.000	0.000	0.000
Curculionoidea	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.221	0.870	9.091	9.922	0.279	0.607	3.704	3.279
Insecta (larva)																
Diptera	29.730	19.947	58.824	2922.155	23.298	18.998	60.000	2537.790	8.628	8.747	72.727	1263.695	66.574	37.373	70.370	7314.753
Odonata	0.000	0.000	0.000	0.000	0.524	3.529	20.000	81.057	0.000	0.000	0.000	0.000	1.671	7.756	14.815	139.666
Plecoptera	1.351	3.998	5.882	31.464	0.000	0.000	0.000	0.000	0.221	0.989	9.091	11.001	5.292	13.099	33.333	613.057
Zooplankton																
Branchiopoda																
Chydorus sphaericus	2.703	0.065	5.882	16.282	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Copepoda																
Naupli	1.351	0.000	5.882	7.950	0.262	0.000	10.000	2.618	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cyclopoida	33.784	0.700	35.294	1217.059	11.257	0.283	50.000	576.991	0.000	0.000	0.000	0.000	0.557	0.010	7.407	4.198
Ostracoda																
Ostracoda	10.811	1.200	23.529	282.608	43.717	5.695	90.000	2,828.187	77.876	12.848	27.273	1,613.897	0.279	0.026	3.704	1.127

Continuation of Table 5.5.

Season >	Autumn				Winter				Spring				Summer			
Prey item	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI
Other inVertebrates																
Amphipoda																
Amphipoda	5.405	11.880	11.765	203.359	3.141	8.391	30.000	345.968	2.876	9.551	18.182	225.956	0.000	0.000	0.000	0.000
Gammaridae	12.162	26.730	47.059	1830.235	8.377	22.376	80.000	2460.219	9.071	30.124	54.545	2137.893	2.507	4.610	22.222	158.156
Bivalvia																
Bivalvia	1.351	0.032	5.882	8.137	3.927	0.113	30.000	121.191	0.000	0.000	0.000	0.000	10.864	0.215	7.407	82.065
Gastropoda																
Gastropoda	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.221	0.082	9.091	2.761	0.279	0.057	3.704	1.245
Acariformes																
Terrestrial type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Aquatic type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.221	0.002	9.091	2.027	3.621	0.016	14.815	53.879
Nematoda																
Short type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	5.014	0.000	11.111	55.711
long type	1.351	0.213	5.882	9.204	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.279	0.037	3.704	1.168
Vertebrates																
Fish larvae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.279	1.639	3.704	7.103
Detritus	NA	35.235	88.235	NA	NA	38.837	100.000	NA	NA	36.402	81.818	NA	NA	33.997	81.481	NA

Table 5.6. N%, V%, FO% and IRI values for all seasons from the Spring habitat.

Season >	Summer				Autumn				Winter				Spring			
	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI
Animalia																
Insecta (adult)																
Diptera	0.667	0.059	4.000	2.904	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.775	1.320	25.000	77.376
Corixidae	0.667	0.137	4.000	3.216	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Curculionoidea	1.333	1.161	8.000	19.953	1.887	1.696	16.667	59.706	0.538	2.404	12.500	36.771	0.000	0.000	0.000	0.000
Insecta (larva)																
Diptera	17.333	3.889	40.000	848.912	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.959	5.544	16.667	141.708
Odonata	4.667	8.657	16.000	213.173	1.887	3.613	16.667	91.659	0.538	5.122	12.500	70.748	0.000	0.000	0.000	0.000
Plecoptera	14.667	14.510	32.000	933.660	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.592	4.889	8.333	45.669
Zooplankton																
Branchiopoda																
Chydorus sphaericus	0.667	0.005	4.000	2.688	18.868	0.157	16.667	317.088	78.495	3.257	12.500	1021.899	42.604	2.874	16.667	757.967
Copepoda																
Naupli	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cyclopoida	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ostracoda																
Ostracoda	13.333	0.495	28.000	387.193	5.661	0.191	50	162.824	5.376	1.025	37.500	240.054	45.562	14.058	66.666	3,442.42
Other inVertebrates																
Amphipoda																
Amphipoda	0.667	0.490	4.000	4.627	3.774	2.863	16.667	110.612	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gammaridae	36.000	26.462	48.000	2998.163	67.925	51.536	83.333	9955.059	13.978	52.772	87.500	5840.664	3.550	21.792	41.667	1055.942
Bivalvia																
Bivalvia	3.333	0.026	12.000	40.317	0	0	0	0	0	0	0	0	0	0	0	0
Gastropoda																
Gastropoda	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.075	0.456	25.000	38.272	0.592	0.408	8.333	8.328

Continuation of Table 5.6.

Season >	Summer				Autumn				Winter				Spring			
	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI	N%	V%	FO%	IRI
Other inVertebrates																
Acariformes																
Terrestrial type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.775	0.587	8.333	19.681
Aquatic type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nematoda																
Short type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
long type	4.000	0.211	12.000	50.534	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Vertebrates																
Fish larvae	2.000	4.704	4.000	26.817	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.592	11.623	8.333	101.786
Detritus	NA	39.192	76.000	NA	NA	39.944	100.000	NA	NA	34.964	100.000	NA	NA	36.905	91.667	NA

5.2.3. Spatial Variation in Diet Composition

The prey items were categorized into different taxonomic groups and their attributes, including the percentage of occurrence (N%), volumetric percentages (V%), frequency of occurrence (FO%), and index of relative importance (IRI), were evaluated. Figure 5.7 presents an analysis of the distribution and relative importance of prey items within the the Stream and Spring sampling locations.

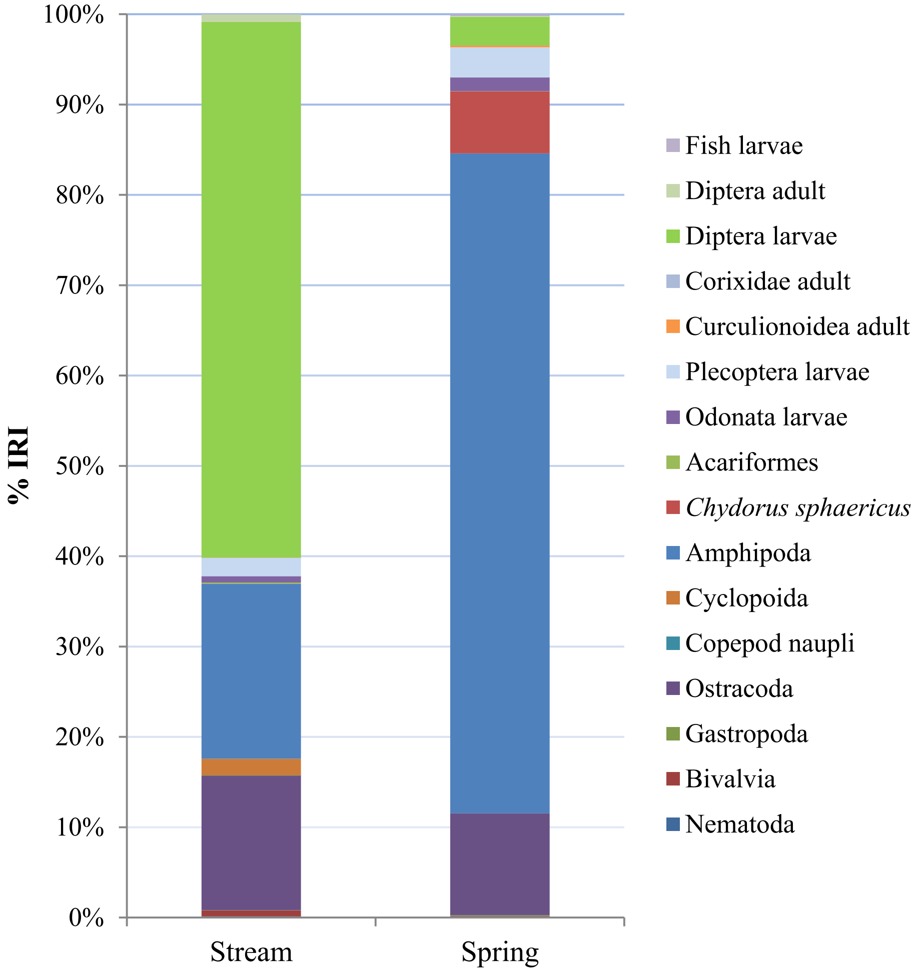


Figure 5.7. IRI Values for the Stream and Spring Habitats

In the Stream, the prey items were dominated by various invertebrate taxa. Amongst the adult insects, Diptera comprised 2.526% of the total occurrences, with a volumetric percentage (V%) of 0.763. Corixidae and Curculionoidea accounted for 0.079% and 0.158% of occurrences, respectively. The highest FO% was observed in Curculionoidea (3.077%), indicating its frequent presence. However, Corixidae exhibited the lowest FO% (1.538%). In terms of IRI, Diptera had the highest index (50.595), followed by Curculionoidea (1.920) and Corixidae (0.206).

Amongst the larval insects, Diptera larvae constituted a substantial portion, contributing to 30.702% of occurrences and 23.376% of volumetric percentages. Odonata larvae and Plecoptera larvae were also noteworthy, constituting 0.631% and 1.657% of occurrences, respectively. The highest FO% was observed in Diptera larvae (66.154%), indicating their prevalence. In terms of IRI, Diptera larvae exhibited a remarkably high index (3577.489), indicating their significant importance in the ecosystem.

Zooplankton in the Stream habitat included various taxa such as *Chydorus sphaericus* and copepods. *Chydorus sphaericus* had a modest presence with N% of 0.158% and V% of 0.004%, yet it displayed a high FO% (1.538%) and IRI (374.776). Copepods, particularly the cyclopoida group, showed N% of 5.525%, V% of 0.130%, FO% of 20.000%, and IRI of 113.094.

The Stream habitat also featured zooplankton of the ostracoda group, with suspended and benthic types. Suspended ostracoda contributed significantly with N% of 39.148%, V% of 4.931%, FO% of 20.000%, and IRI of 881.574. Benthic ostracoda displayed N% of 2.526%, V% of 0.204%, FO% of 6.154%, and IRI of 16.795.

Other invertebrates included amphipods and bivalves. Amphipods, particularly Gammaridae, demonstrated considerable presence with N% of 7.182% and V% of 17.913%. Gammaridae had the highest FO% (43.077%) and IRI (1081.030) in this category. Bivalves and gastropods exhibited comparatively lower presence, with Bivalvia having N% of 4.341% and Gastropoda N% of 0.158%.

The species contributions, similarities, and dissimilarities within the "Spring" and "Stream" habitat groups using the Canonical Analysis of Principal Coordinates (CAP) are given in Figure 5.8. The dataset comprising volume data, was subjected to one-way analysis to elucidate the ecological patterns present in the two habitat groups.

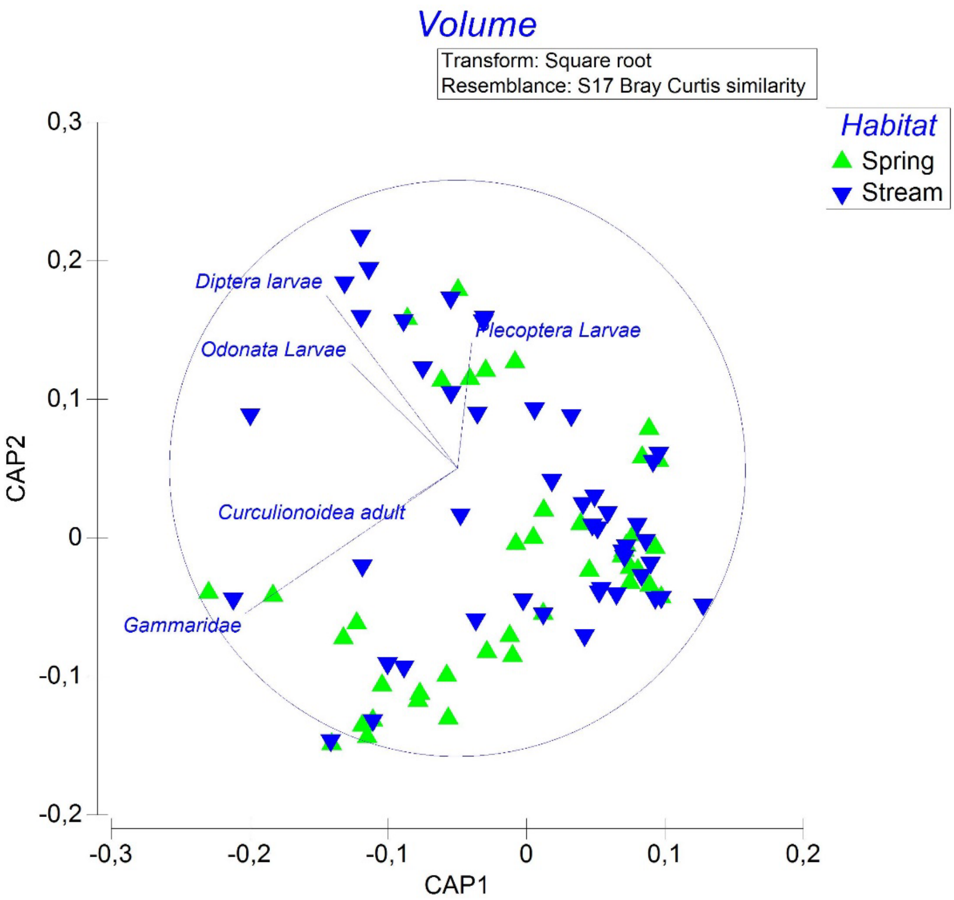


Figure 5.8. Canonical Analysis of Principal Coordinates (CAP) Ordination of the Examined Stomachs of *G. insuyanus* between Stream and Spring Habitats

In the SIMPER analysis of the 'Spring' habitat group, the average similarity within the group was determined to be 27.55%. The most significant species contributing to this similarity were Gammaridae, with an average abundance of 1.98 and a contribution to the similarity of 81.16%. Ostracoda of the suspended type and Diptera larvae also contributed significantly, with cumulative percentages of 88.11% and 92.98%, respectively. Additionally, the 'Stream' habitat group exhibited an average within-group similarity of 29.33%. Diptera larvae were the primary contributors, accounting for 61.56% of the similarity, with an average abundance of 1.65. Following were Gammaridae, which had an average abundance of 1.21 and a contribution of 29.21% to the similarity, resulting in a cumulative contribution of 90.77%.

The Spring and Stream groups had an average dissimilarity of 78.11%. The main contributors to this dissimilarity were the Gammaridae species, with average abundance of 1.98 in 'Spring' and 1.21 in 'Stream', accounting for 32.47% of the dissimilarity. Following this, Diptera larvae contributed 24.11%, and were succeeded by Plecoptera larvae, Ostracoda of the suspended type, and other species, cumulatively amounting to 56.58%, 74.46%, and 81.01%, respectively. Amphipods, larvae of Actinopterygii, and adult Diptera were also identified as contributors, with their combined contribution amounting to 90.29%.

5.2.4. Seasonal Variation in Diet Composition

Vertebrates, specifically fish larvae, were present in the spring habitat. Fish larvae displayed N% ranging from 0.079% to 0.636%, while detritus had substantial N% values and the highest FO% and V% values, suggesting their significant presence. The seasonal variation in IRI values of prey items are given in Figure 5.9. The data, organized into categories based on animal taxa, provides insights into the preferences and patterns of prey consumption during distinct seasons.

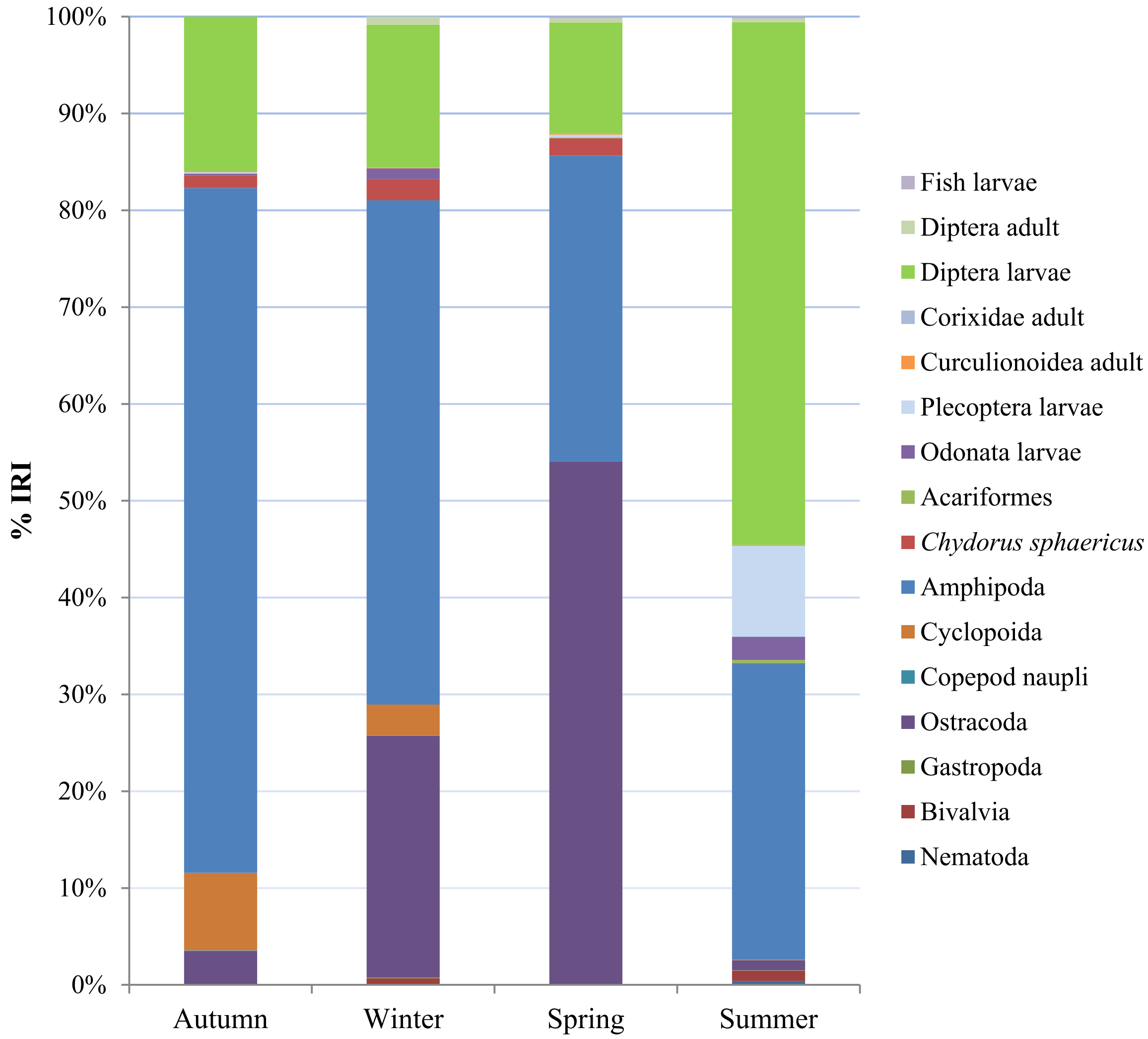


Figure 5.9. IRI Values of Food Items Recorded Across the Four Seasons

In the category of adult insects, Diptera demonstrated a significant presence across all seasons, with the highest occurrence during summer (3.697%). Corixidae showed negligible occurrence, while Curculionoidea displayed notable variation, with the highest frequency observed during winter (1.144%).

Amongst insect larvae, Diptera experienced a substantial presence throughout the seasons, with the peak occurrence in autumn (17.323%). Odonata and Plecoptera also exhibited season-specific preferences, with the highest prevalence of Odonata during winter (2.438%) and Plecoptera during summer (1.645%).

Within the zooplankton category, *Chydorus sphaericus* displayed a considerable presence during autumn (9.449%), while Cyclopoida exhibited a distinct peak in occurrence during winter (19.685%). Ostracoda revealed a unique pattern with suspended type dominating during winter (66.345%) and benthic type having a presence only in autumn (0.787%).

Amphipoda, particularly Gammaridae, emerged as prominent prey items in this category. Gammaridae showed the highest prevalence during winter (43.468%), while Amphipoda had a more even distribution across seasons. Bivalvia and Gastropoda exhibited varying preferences, with Bivalvia occurring predominantly during spring (8.065%) and Gastropoda showing a notable presence in winter (0.352%).

Acariformes, divided into terrestrial and aquatic types, displayed different consumption patterns. The terrestrial type demonstrated a preference for spring (0.483%), while the aquatic type showed an increased presence during summer (0.161%).

Short type nematodes exhibited notable presence during spring (3.103%) and summer (4.839%). Long type nematodes had a relatively consistent occurrence, with a peak in spring (1.207%).

Fish larvae showed a preference for spring (1.956%), while detritus consumption remained consistent across all seasons.

Canonical Analysis of Principal Coordinates (CAP) ordination of the examined stomachs of *G. insuyanus* is depicted in Figure 5.10. SIMPER analysis resulted in consistent outcome with the vector lines in CAP.

In the SIMPER analysis, the within-group average similarity for the 'Summer' (Su) season was 25.85%, with Diptera larvae and Gammaridae being the primary contributors, at 43.13% and 38.02% respectively. The 'Autumn' (Au) group showed a higher average similarity of 28.46%, predominantly due to Gammaridae at 62.83%. The 'Winter' (Wi) season had the highest within-group similarity of 34.19%, with Gammaridae making the largest contribution of 83.66%. The 'Spring' (Sp) season had the lowest average similarity at 20.71%, with Gammaridae again contributing the most at 53.23%.

Between-group average dissimilarities were as follows: 'Summer' and 'Autumn' at 74.87%, 'Summer' and 'Winter' at 75.87%, 'Autumn' and 'Winter' at 70.00%, 'Summer' and 'Spring' at 78.16%, 'Autumn' and 'Spring' at 75.20%, and 'Winter' and 'Spring' at 73.62%. Gammaridae consistently appeared as a significant contributor to dissimilarity between all seasonal comparisons, with contributions ranging from 29.29% to 39.53%. Other species like Diptera larvae, Plecoptera larvae, and Ostracoda Suspended type also made notable contributions to the dissimilarity across different seasonal comparisons.

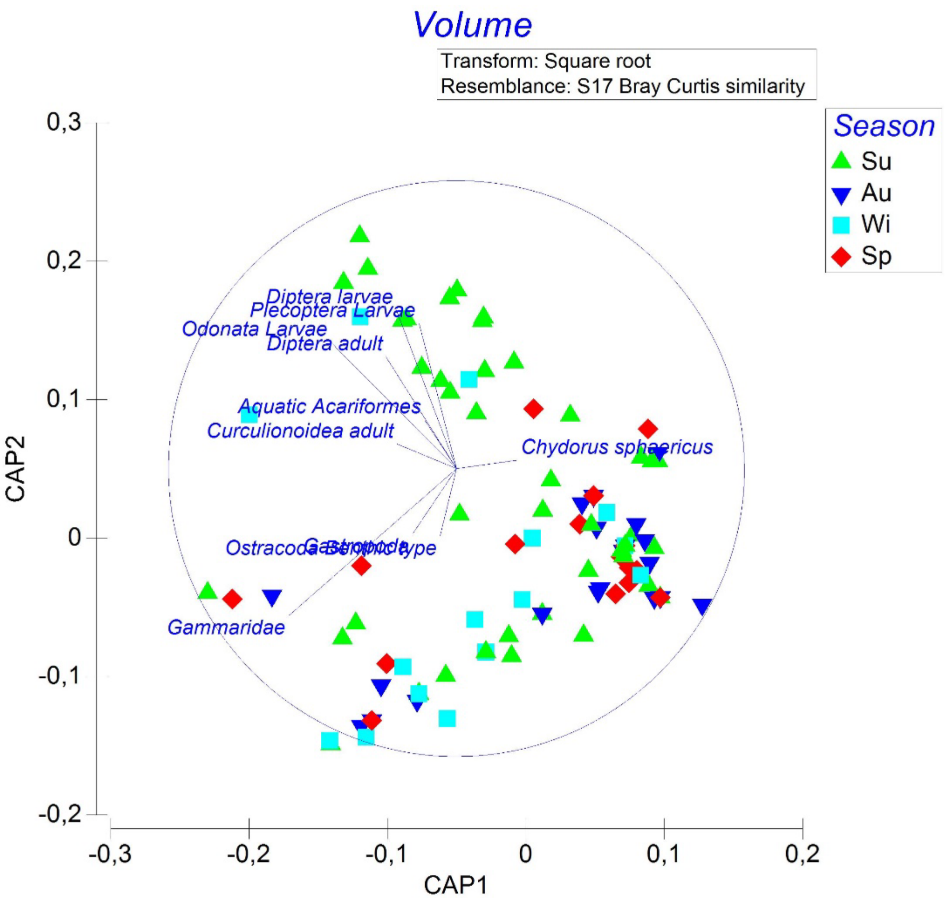


Figure 5.10. Canonical Analysis of Principal Coordinates (CAP) Ordination of the Examined Stomachs of *G. insuyanus* Across the Seasons

5.2.4. Intraspecific Variation in Diet Composition

IRI food item values for the mature and immature stages of *Gobio insuyanus*, providing insights into the differing feeding preferences between the two life stages are given in Figure 5.11.

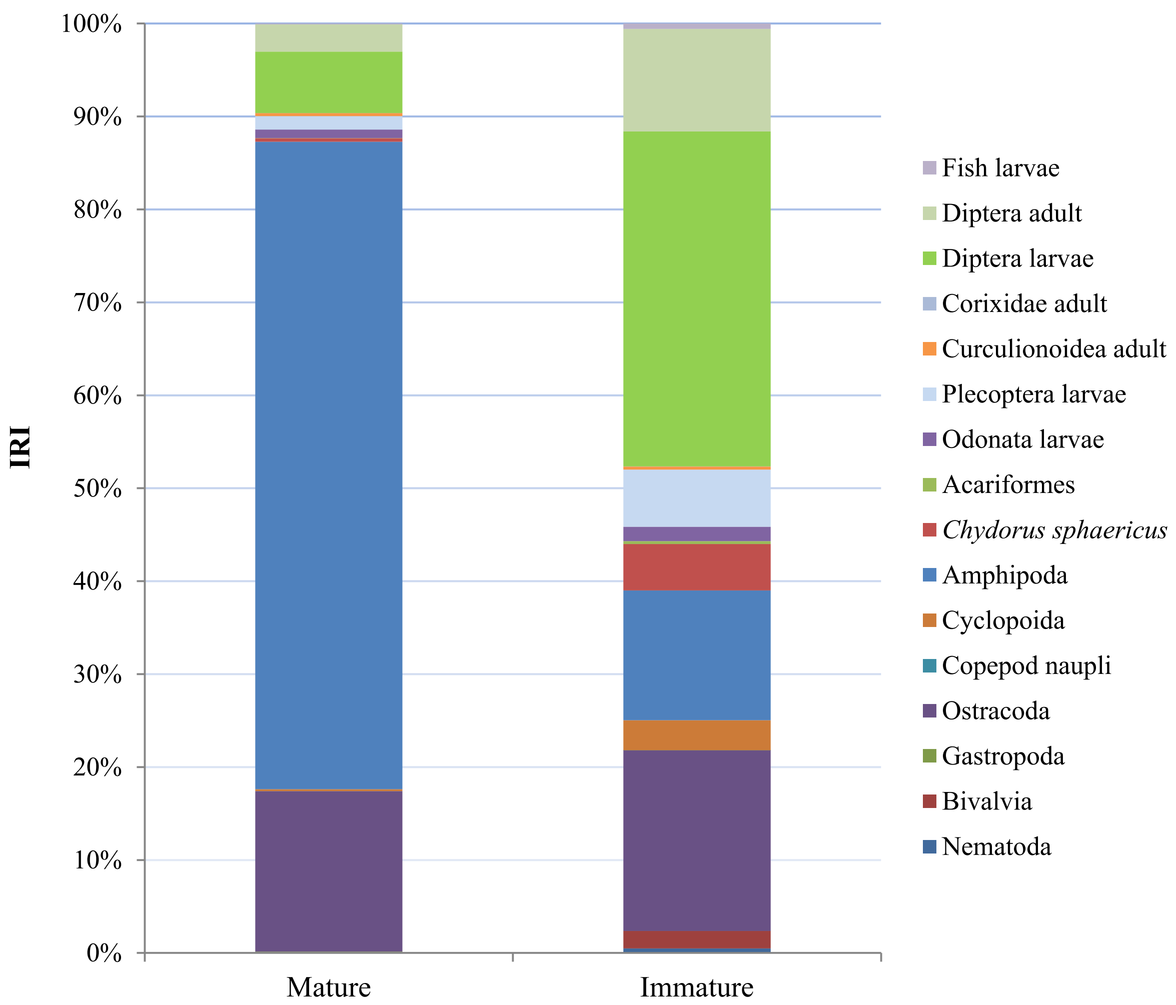


Figure 5.11. IRI values for the Mature and Immature Stages of *G.insuyanus*

In mature *Gobio insuyanus*, the dominant prey item was the Gammarid type Amphipod, with the highest Index of Relative Importance (IRI) at 4486.951. This prey type also led in frequency of occurrence (FO%) at 81.818% and in volumetric contribution (V%) at 34.139%. Notably, mature fish displayed a more diversified range of prey items with high IRI values, including the suspended type Ostracoda (IRI = 1110.239). Detritus also constituted a significant part of the diet, accounting for 38.507% of the diet by volume.

In contrast, immature individuals exhibited a different dietary focus. The highest IRI was for Dipteran larvae, at 1430.169, with an FO% of 54.545% and a V% of 26.087%. Unlike mature fish, immature individuals had a narrower range of high IRI values. Detritus was also present but slightly less so than in mature fish, comprising 33.681% of the diet by volume. Fish larvae had a slightly higher IRI in immature fish (23.048) compared to mature fish (4.829).

The CAP ordination plot (Figure 4.12) shows the correlation between *G. insuyanus* maturity stages and their diet composition. Mature *G. insuyanus* are clustered towards the negative end of the CAP1 axis, with a strong preference for Gammaridae, indicating a higher prevalence of this prey in mature individuals. Immature individuals, on the other hand, are widely dispersed across the plot, suggesting a more diverse diet. However, there is a significant clustering around Diptera larvae, Odonata larvae and Plecoptera larvae, which are closer to the centre of the ordination space.

The overall distribution of points suggests some dietary overlap between both developmental stages, yet specific prey species such as Gammaridae and Curculionoidea adults exhibit a stronger association with mature individuals, as they are positioned further along the CAP1 axis. The prey items are distributed along the CAP2 axis, indicating that this axis is less effective in distinguishing dietary composition between various maturity stages. The ellipses enclosing most data points for each group show no distinct separation, implying that although there are certain patterns in prey preference, dietary composition is not entirely differentiated based on maturity stage.

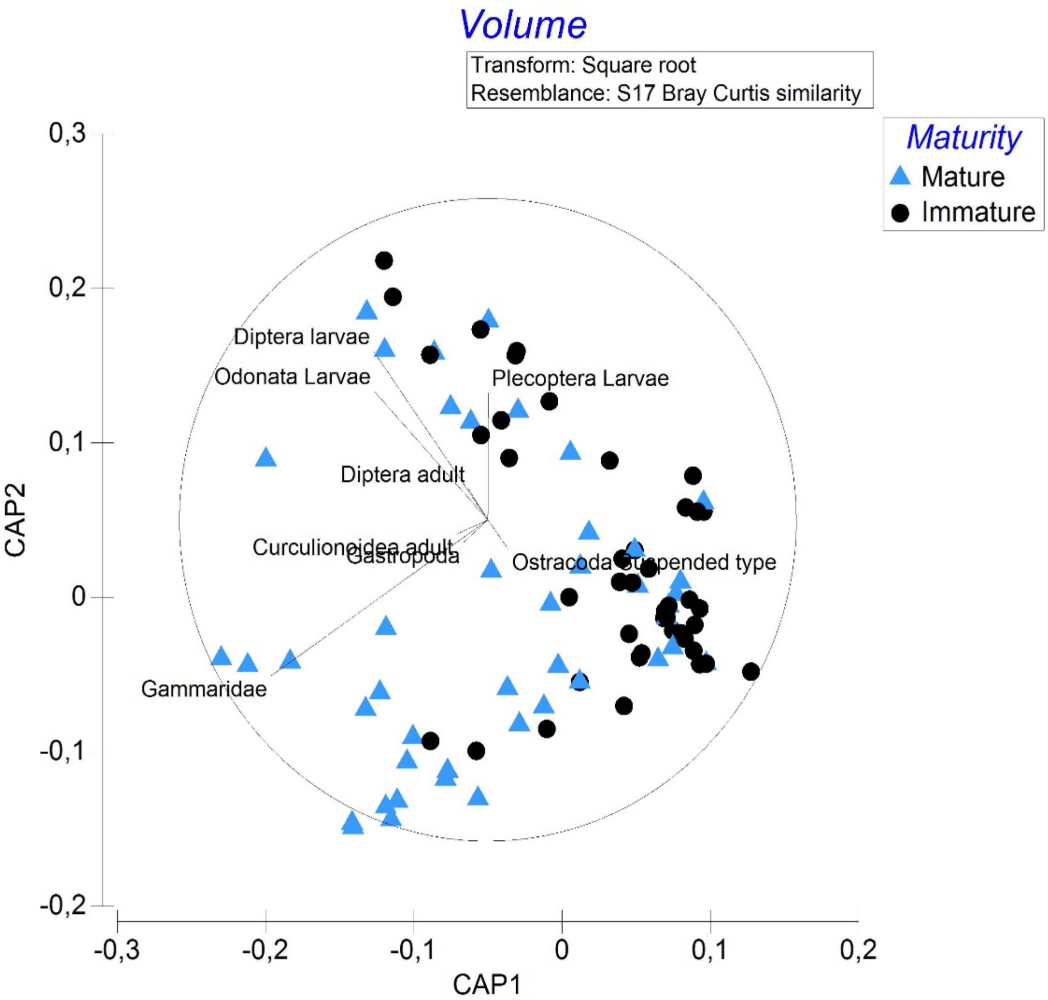


Figure 5.12. Canonical Analysis of Principal Coordinates (CAP) Ordination of the Examined Stomachs of *G. insuyanus* Between Maturity Stages

The SIMPER analysis showed that the 'mature' group of *G. insuyanus* had an average within-group similarity of 37.02%. The group was mainly characterized by Gammaridae, which had an average abundance of 2.53 and contributed significantly to the group's similarity with 79.80%. Following were Diptera larvae, which contributed 13.90% and led to a total similarity of 93.70%.

In contrast, the 'Immature' group exhibited a lower average similarity of 19.97%. Diptera larvae were the primary contributors in this group with an average abundance of 1.02 and a contribution to similarity of 51.15%. Following Diptera were the Gammaridae and Ostracoda Suspended types with contributions of 25.41% and 11.62%, respectively. Together, they contributed 88.18% towards the similarity. Plecoptera larvae also offered a degree of contribution, leading to a cumulative similarity value of 94.75%.

The mature and immature groups showed an average dissimilarity of 78.12%. Gammaridae showed the greatest contribution to dissimilarity at 38.05% between the two groups, displaying notable variations in average abundances (2.53 in 'Mature' and 0.65 in 'Immature'). Additionally contributing significantly to dissimilarity were Diptera larvae, Plecoptera larvae, and Ostracoda Suspended type, resulting in a cumulative contribution of 75.36%. Species such as Odonata larvae, Amphipoda, and Curculionoidea adults were less contributive, resulting in a cumulative dissimilarity of 91.12%.To investigate the influence of Habitat (Ha), Season (Sea), Sex, and Maturity (Ma) on the ecological variables of interest, a Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted. Table 5.7 displays the results. The analysis was based on a Bray-Curtis similarity matrix of prey volume data and utilized 999 permutations.

Table 5.7. Results of PERMANOVA to assess the influence of Habitat (Ha), Season (Sea), Sex, and Maturity (Ma)—and their interactions on the diet composition of *G. insuyanus*. Degrees of Freedom (df), Sums of Squares (SS), Mean Squares (MS), Pseudo-F ratios, and permutation-derived *p*-values (P(perm)).

Source	**Degrees of Freedom (df)**	**Sums of Squares (SS)**	**Mean Squares (MS)**	**Pseudo-F**	**P-value (P(perm))**	**Significance**
Habitat (Ha)	1	19849	19849	9.0628	0.001	Significant
Season (Sea)	3	14260	4753.5	2.1703	0.01	Significant
Sex	1	2039.4	2039.4	0.93117	0.452	Not Significant
Maturity (Ma)	1	15943	15943	7.2795	0.001	Significant
Ha x Sea	3	11427	3808.9	1.7391	0.051	Not Significant
Ha x Sex	1	3971.9	3971.9	1.8135	0.076	Not Significant
Ha x Ma	1	4013.9	4013.9	1.8326	0.116	Not Significant
Sea x Sex	3	8813.6	2937.9	1.3414	0.173	Not Significant
Sea x Ma	3	5446.1	1815.4	0.82886	0.66	Not Significant
Sex x Ma	1	1972.9	1972.9	0.90078	0.476	Not Significant
Ha x Sea x Sex	3	5405.6	1801.9	0.8227	0.649	Not Significant
Ha x Sea x Ma	3	11905	3968.4	1.8119	0.038	Significant
Ha x Sex x Ma	1	2871	2871	1.3108	0.248	Not Significant
Sea x Sex x Ma	2	2794.1	1397.1	0.63787	0.752	Not Significant

The PERMANOVA results indicated significant effects of habitat (Ha), season (Sea) and maturity (Ma) on the diet composition of *G. insuyanus* with p-values of 0.001, 0.01 and 0.001 respectively. Diet composition was not affected significantly by Sex ($p = 0.452$). Although it did not meet the standard threshold for statistical significance, the interaction between habitat and season was marginally significant ($p = 0.051$), suggesting a possible combined effect on diet composition. All other two- and three-way interactions, such as Habitat by Sex, Habitat by Maturity, Season by Sex, Season by Maturity, Sex by Maturity, Habitat by Season by Sex, Habitat by Season by Maturity, and Habitat by Sex by Maturity, did not show any statistical significance. However, the interaction between Habitat, Season, and Maturity was significant ($p = 0.038$), suggesting that the combined influence of these three factors had a significant impact on the diet composition of *G. insuyanus.*

5.3. FEEDING ECOLOGY

5.3.1. Diet Diversity and Niche Breadth

In order to further analyse the feeding ecology of *G.insuyanus*, further statistical analysis was preformed. Table 5.8 displays the Simpson's index, the Shannon-Wiener index and the evenness value for *G. insuyanus* for the different factors.

Table 5.8. The diet diversity profile of *Gobio insuyanus* across habitat, sex (F, Female; M, Male), season (Au, Autumn; Sp, Spring; Su, Summer; Wi, Winter) and ontogeny factors.

	Spring	**Stream**	**F**	**M**	**Au**	**Sp**	**Su**	**Wi**	**Immat.**	**Mat.**
Taxa number	18	17	17	16	12	14	16	12	17	17
Simpson 1-D	0.56	0.76	0.67	0.75	0.48	0.72	0.74	0.69	0.77	0.65
Shannon H	1.31	1.72	1.59	1.61	1.07	1.59	1.55	1.53	1.79	1.50
Evenness_e^ H/S	0.21	0.33	0.29	0.31	0.24	0.35	0.30	0.38	0.35	0.26

The diet diversity of *Gobio insuyanus* differed based on habitat, season, sex, and maturity stage. Specifically, the diversity was higher in the 'Stream' habitat as compared to the 'Spring' habitat. The Simpson 1-D index for the 'Stream' was 0.76 in comparison to 0.56 for the 'Spring'. Additionally, the Shannon index for the 'Stream' was 1.72 while that for the 'Spring' was 1.31. Seasonal variations had an effect on dietary diversity, with 'Spring' and 'Summer' showing similar Simpson indices (0.72 and 0.74 respectively) and Shannon indices (1.59 and 1.55 respectively). 'Autumn' recorded the lowest diversity, with a Simpson 1-D of 0.48 and Shannon index of 1.07, while 'Winter' displayed intermediate diversity levels (Simpson 1-D of 0.69 and Shannon index of 1.53). Sexual differences were observed; females (F) had a Simpson 1-D of 0.67 and a Shannon index of 1.59, while males (M) had a slightly higher Simpson 1-D of 0.75 and a comparable Shannon index of 1.61. In terms of maturity, immature individuals (Immat.) revealed a higher diversity (Simpson 1-D of 0.77, Shannon index of 1.79) than mature individuals (Mat.), which had a Simpson 1-D value of 0.65 and a Shannon index of 1.50. Evenness varied among the factors studied, with higher evenness values observed in 'Stream' habitats, during the 'Summer' and 'Spring' seasons, and in immature individuals. This suggests a more equitably distributed species in their diets compared to their counterparts. The H/S ratio, which represents species diversity as a proportion of the number of taxa, followed a similar pattern. Figure 5.13 presents the, Shannon's Diversity Index (*H*) is plotted for various categories: Spring, Stream, F, M, Au, Sp, Su, Wi, Immature, and Mature.

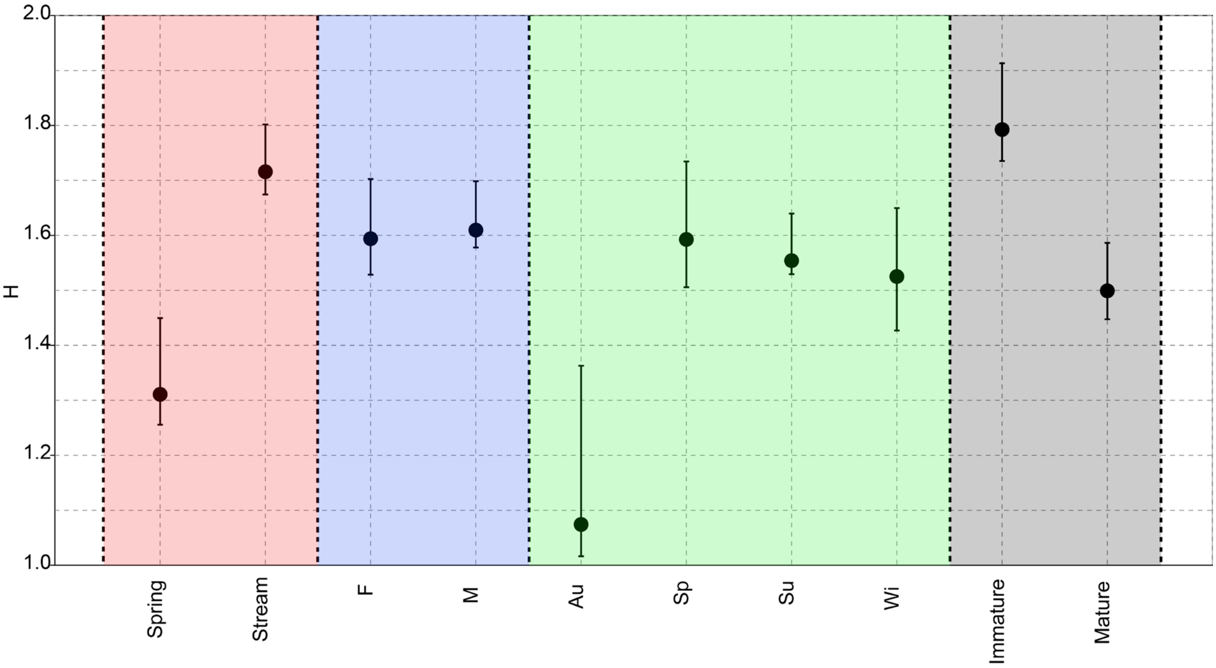


Figure 5.13. The Shannon–Weiner Indices for the Various Factors Analysed for *G. insuyanus*.

Figure 5.14 displays the distribution of bootstrapped Levin's Niche Breadth indices for the various factors. The analysis of Levin's Niche Breadth indices indicated variability in the dietary breadth of *Gobio insuyanus* across different factors. 'Spring' and 'Stream' habitats demonstrated a wide range of dietary breadth, with 'Stream' habitats exhibiting slightly higher median values. Among the sexes, females (F) had a broader dietary niche than males (M). Seasonal analysis showed 'Summer' (Su) and 'Spring' (Sp) with a wider dietary breadth compared to 'Autumn' (Au) and 'Winter' (Wi), which both had narrower niches. When comparing maturity stages, immature individuals displayed a broader dietary niche than mature ones, suggesting a more diverse diet in immature *G. insuyanus*. The spread of the data points indicated variability within each category, with some overlap between them.

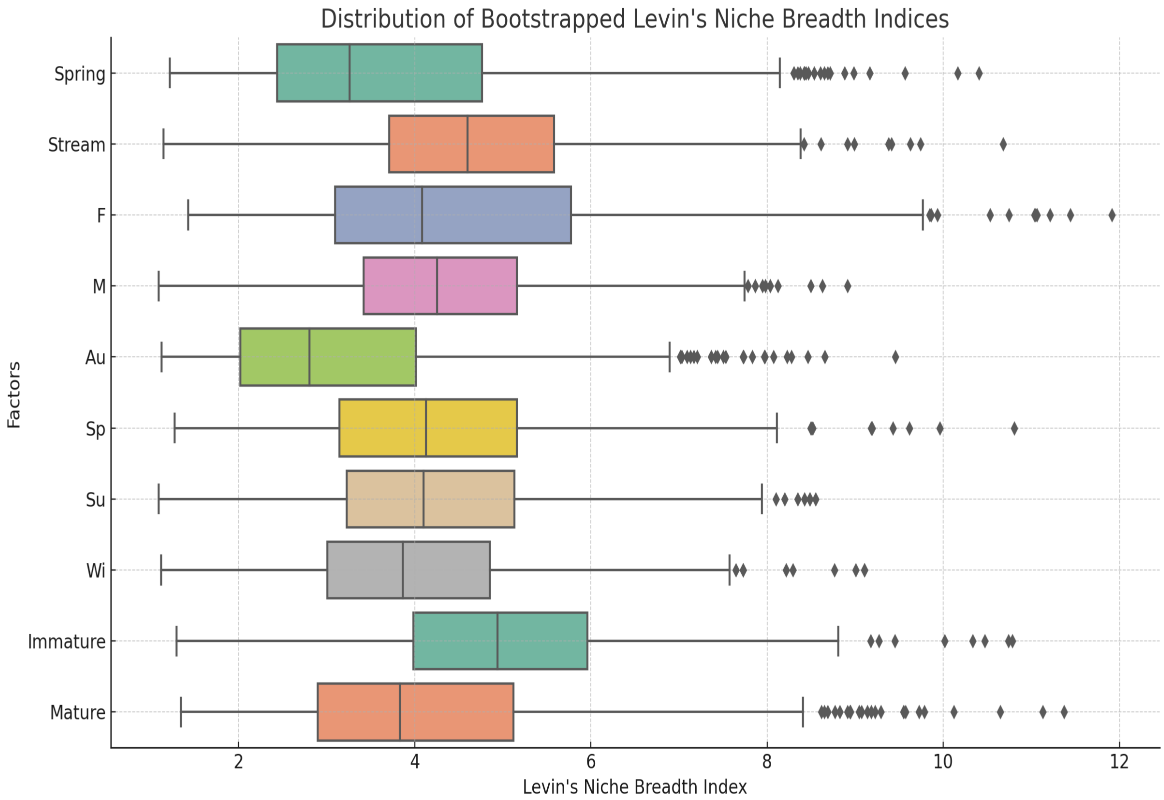


Figure 5.14. Distribution of Bootstrapped Levin's Niche Breadth Indices Across Studied Factors

The correlation matrix for bootstrapped Levin's Niche Breadth indices for *Gobio insuyanus* indicates a strong positive correlation between females during spring and their similar niche breadth. In contrast, mature individuals exhibit a considerably narrower dietary niche in spring, as indicated by a strong negative correlation with season. In autumn, mature individuals exhibit a more specialised diet, with 'autumn' and 'mature' also showing a strong negative correlation. Immature individuals in Stream habitats are observed to have a broad range of dietary choices, as evident from a strong positive correlation between these two factors. Seasonal comparisons indicate a positive correlation between 'Spring' and 'Females', and between 'Summer' and 'Winter', suggesting similar niche breadths within these groupings. Correlation Matrix of Bootstrapped Levin's Niche Breadth Indices is displayed in Figure 5.15.

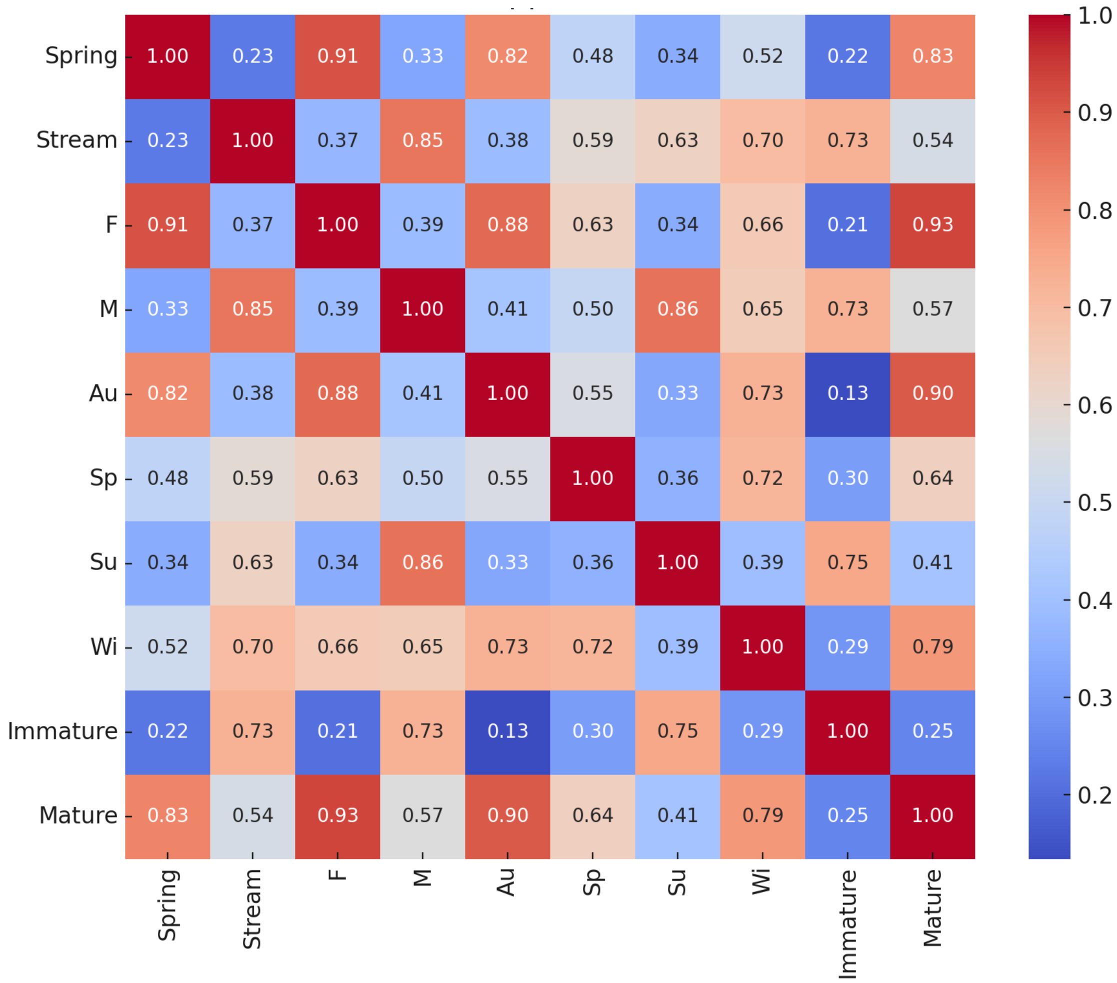


Figure 5.15. Correlation Matrix of Bootstrapped Levin's Niche Breadth Indices

5.3.2. Trophic Levels

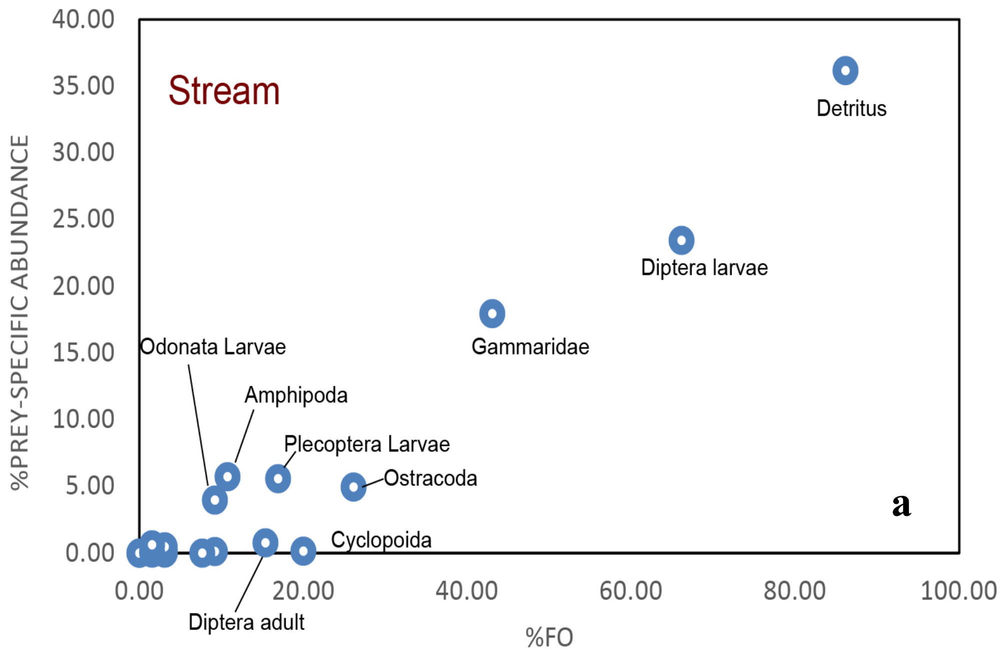
Table 5.9 diplays the trophic levels calculated for each factor, and their associated standard errors. The values of trophic leves without detritus ranged between 3.25 and 3.35 suggesting *G. insuyanus* is at the level of carnivores. When detritus is included, the values naturally decrease (ranging from 2.78 to 2.88), as detritus has the same trophic level as plant material. As the content of detritus in our sample is unknown, the values derived from computations with the exclusion of detritus would be more accurate.

Table 5.9. Trophic Levels for Various Factors With and Without the Food Item Detritus

	Without detritus		**With detritus**	
	Trophic Level	**s.e.**	**Trophic Level**	**s.e.**
Spring	3.32	0.35	2.82	0.21
Stream	3.27	0.22	2.81	0.14
F	3.34	0.3	2.83	0.19
M	3.25	0.24	2.80	0.15
Au	3.27	0.38	2.78	0.23
Sp	3.35	0.28	2.86	0.18
Su	3.29	0.24	2.81	0.15
Wi	3.27	0.28	2.79	0.18
Immature	3.32	0.21	2.88	0.14
Mature	3.28	0.31	2.79	0.19

5.3.3. Feeding Strategy

The modified Costello's graphical analysis of the feeding strategy of *Gobio insuyanus* revealed a distinct dietary pattern across the habitats studied. Specifically, some prey types exhibited a considerable prey-specific abundance despite a low frequency of occurrence (%FO). This pattern suggests the presence of specialised subgroups within the G. insuyanus population that have a preference for these prey items. In the 'Stream' environment, the occasional but substantial consumption of certain prey items, such as dipteran larvae and detritus, suggests that a subset of individuals may have specialised feeding habits, preferentially targeting these resources when they are available. While not frequently consumed by the entire population, the importance of these prey items in the diet of these specialised feeders is considerable. The data from the 'Spring' habitat supports this analysis. The prevalence of Gammaridae in the diet, indicated by a high prey-specific volume and %FO, suggests a widespread preference for this type of prey among the population. Nevertheless, other prey categories with substantial volume but lower incidence, such as Odonata larvae, may indicate specialized feeding patterns among a smaller proportion of the population. These findings highlight the presence of dietary specialization in the *G. insuyanus* population, with specific individuals or groups displaying distinct feeding preferences that considerably contribute to their nutritional intake. This specialization can impact the ecological dynamics of the habitats, affecting prey populations and the role of *G. insuyanus* within the trophic system.



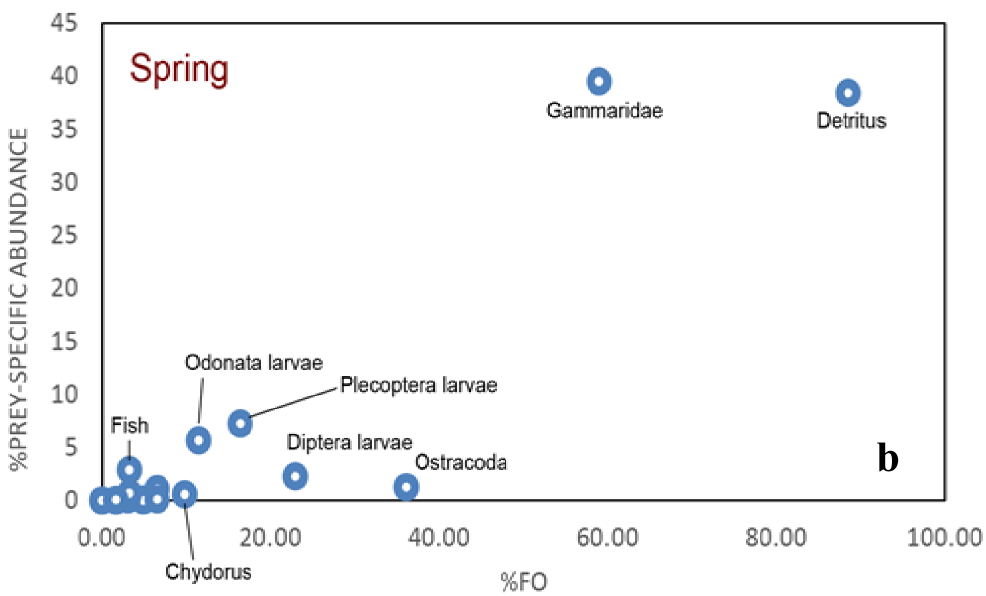


Figure 5.16. Costello's Prey-specific Abundance Graph Displaying the (a) Stream and (b) Spring locations

5. DISCUSSION

The length distribution *of Gobio insuyanus* reveals a wide range of fork lengths, from immature individuals to mature males and females. The smallest individual, an immature one, measured a mere 16.61 mm, emphasizing the early stages of development where gender determination is challenging. In contrast, the largest individual, a female, reached a substantial size of 145.44 mm. This disparity in size showcases the growth potential of the species. The clustering of fork lengths provides insights into the developmental stages of *Gobio insuyanus*. Immature individuals predominantly fall within the shorter fork length range, while mature males and females exhibit distinct size clusters.

The weight distribution of *Gobio insuyanus* offers a comprehensive understanding of the species' growth patterns and potential ecological roles. The observed weight range, from a mere 0.049 grams for immature individuals to a substantial 54.221 grams for mature females, showcases the species' growth trajectory. The fact that the lightest individual, an immature one, weighed only 0.049 grams, highlights the early developmental stages where not only is gender determination challenging, but the individuals are also potentially more vulnerable to predation and environmental factors. In contrast, the heaviest individual, a female, weighing 54.221 grams, indicates the species' potential to attain significant biomass. When comparing these findings with a previous study conducted by Ergönül *et al.* [10], their sampled *Gobio insuyanus* parameters were similar. They recorded a fork length range of 4.4cm – 16.1cm, which lies quite close to the fork length range of 1.46 cm – 13.17 cm recorded in this study. The range given by the Ergönül *et al.* was based upon 53 individuals sampled, with this study having measured 295 individuals, providing a more accurate range of fork lengths presented by the population [10] . Another study conducted by Şenyiğit & Mazlum [11] for her Msc thesis involved collecting measurements from 13 different *Gobio* species, including *Gobio insuyanus*. While only measuring 8 individuals, they recorded a total length range of 7 – 10.5cm. This study's total length range is dissimilar to the current study's total length range, which lies between 1.75cm – 15.2cm, which might be due to the single sampling period and low number of individuals sampled in their study [11]. A technical contribution by Erk'akan *et al.* [9] collected the physical parameters of five cyprinid species found in Turkey, with one of the species sampled being *Gobio insuyanus.* Their findings give a total length range of 6.9cm – 13.6 cm, within a sample size of 18 individuals.

In the previously mentioned study by Ergönül *et al.*, [10] weight measurements were taken of *Gobio insuyanus*. The weight range given in that study put a range of 0.9g – 57.2g, with this study recording a range of 0.05g – 54.22g. While quite close, any differences in the obtained weights of this study might be due to the increase number of samples collected [10].

The age distribution *of Gobio insuyanus* provides crucial insights into the population structure and dynamics of this species. The dominance of younger age groups, particularly age group 0 and age group I, suggests a robust recruitment rate, indicating successful reproduction and survival of juveniles. The presence of 64 individuals in age group I, making it the most prominent age group, underscores the species' potential to reach maturity and contribute to future generations. However, the decreasing number of individuals in subsequent age groups, from age group II to age group V, might indicate natural mortality, predation, or other ecological factors affecting the survival of older age classes. The smaller numbers in age groups III, IV, and V highlight the challenges faced by *Gobio insuyanus* as they age, which could be due to increased predation, competition, or environmental stressors. The lack of specific age distribution studies conducted on *Gobio insuyanus* allows us to compare with other *Gobio* species endemic to Turkey. A 2006 study was conducted by Özdemir [12] on the growth parameters of the species *Gobio gymnostethus,* located in the Melendiz Stream. Their age distribution was different in comparison, with the age V category making up 67.3% of the total 544 individuals they sampled, followed by age IV at 12.2% and age III at 11.7%. While not comparing specifically *Gobio insuyanus*, the Melendiz Stream is a considerably bigger habitat then the Insuyu Stream, allowing for a larger population of *Gobio gymnostethus*, which will result is a considerably different population age dynamic [12].

The sex distribution of *Gobio insuyanus* provides valuable insights into the reproductive dynamics and potential population sustainability of this species. The presence of a higher number of females (154 individuals) compared to males (94 individuals) suggests a female-biased sex ratio as 1.64:1 in this study. Such a distribution can have implications for reproductive success, as a higher number of females can potentially lead to increased egg production and, consequently, a higher number of offspring.

The presence of 47 immature individuals, whose sex could not be determined due to underdeveloped gonads, indicates a healthy recruitment rate, suggesting that the species is successfully reproducing in its habitat. The immature individuals represent the future breeding population, and their survival is crucial for the continuity of the species.

The length-weight relationship (LWR) is a pivotal tool in fisheries biology, offering insights into the growth patterns and overall health of fish populations. For *Gobio insuyanus*, the comparison between the Sping and Stream samples showed no significant difference in the 'b' values, suggesting consistent growth patterns across different habitats. In this study the combined length-weight relationship equation for the entire population, b value is found to be 3.27, indicates positive allometric growth, where the weight of the fish increases at a faster rate than its length as it grows [46] . Erk'akan *et al*. in their study found the 'b' value to be 3.13 while Ergönül *et al*. found the 'b' value for *G. insuyanus* to be 2.92, [9,10]. Şenyiğit and Mazlum calculated the 'b' value of 2.73 [11]. While these three 'b' values were dissimiliar to the 'b' value found in this study, we have to take into consideration the sample sizes used in the three studies. Sample sizes of 18, 53, 8 individuals were utilised in the studies of Erk'akan *et al*., Ergönül *et al*. and Şenyiğit and Mazlum respectively. In comparison to this study, the number of individuals sampled was 295, a far larger sample size. A larger sample size would reflect a calculated 'b' value closer to the actual value, of which this study has provided.

The diet composition of *G.insuyanus* reveals a diverse range of prey items, reflecting the adaptability and ecological versatility of the species. The presence of both adult and larval forms of Insecta, as well as a variety of zooplankton and other invertebrates, indicates a broad dietary spectrum across all *G.insuyanus* samples analysed. The diet composition of *G.insuyanus* in both Stream and Spring habitats reveals a diverse range of prey items, reflecting the adaptability and opportunistic feeding behavior of the species. In the Stream habitat, invertebrate taxa, particularly adult and larval forms of Diptera, dominate the diet. The significant presence of Diptera larvae in the Stream habitat, with an impressive average similarity of 18.05, further underscores their importance in the diet of *G.insuyanus* from this habitat. In contrast, the Spring habitat shows a pronounced influence of Gammaridae, contributing significantly to the overall resemblance within the habitat group. The observed dissimilarity between the two habitats, with an average of 78.11, suggests distinct dietary preferences or availability of prey items in these habitats. A 1972 study was conducted on the species *Gobio gobio* in Irish fresh waters [16], interestingly their data was sampled from two different location types, river and resevoir. The river samples were dominated by the gammarids, nymphs of Ephemeroptera, larvae of caddis and chirononiids, and molluscs. In contrast, the reservoir data presented a dominance in Cladocera and copepods, with chironomids also showing a large percentage of the *Gobio gobio* sampled from these resevoir locations. While an outright comparison is imposible between *Gobio insuyanus* and *Gobio gobio*, there are similar trends regarding the dominance of both Dipteran larvae (analogues to the lower taxonomic level of chironomids provided in the Irish study) and gammarids. This study also recorded a large component of detritus within the stomach of the sampled gudgeons, which the author proposes was mainly sourced from the digestive tracts of the prey items of *Gobio gobio*, while I propose the addition of accidental ingestions due to the gudgeons bottom feeding behaviour [16]. This fact is further supported by the presence of detritus in all of the stomachs sampled in this study, in both maturity stages and sampling locations.

The diet composition of *G.insuyanus* exhibits pronounced seasonal variations, reflecting the dynamic nature of prey availability and possibly the adaptive feeding strategies of the species. Diptera, both adult and larval forms, consistently emerged as a dominant prey item across all seasons, with a peak occurrence in summer. The significant presence of Gammaridae, especially during winter, underscores their importance in the diet of *G.insuyanus*.

The Bray Curtis similarity metric analysis further elucidates the dietary patterns across seasons. The distinct average similarities and dissimilarities between seasons indicate the influence of environmental factors, prey availability, and possibly interspecific competition on the diet of *G.insuyanus*. The significant contribution of Diptera larvae and Gammaridae to the overall resemblance within and between seasons further emphasizes their dietary importance. The dietary preferences of *G. insuyanus* undergo significant shifts as the fish transition from the immature to mature stages. Mature individuals predominantly feed on the Gammarid type Amphipod, which not only has the highest Index of Relative Importance (IRI) but also leads in frequency of occurrence (FO%) and volumetric contribution (V%). This finding is consistent with the observations of Bolnick *et al.* [50], who noted that mature fish often exhibit a preference for specific prey types due to their larger size and evolved feeding mechanisms [51,52]. The Bray-Curtis similarity index further underscores the dietary differences between mature and immature fish. While Gammaridae dominates the diet of mature fish, Diptera larvae emerge as the primary food source for immature individuals, which dominate their diet in terms of IRI, FO%, and V%. The significant dissimilarity between the two groups reflects the adaptive feeding strategies employed by *Gobio insuyanus* at different life stages, with smaller and younger individuals having access to smaller food items such as dipteran larvae, while gammarids, being more motile and often larger forming a rarer food item for them to feed upon. It is important to note that this dissimilarity in food item choice demonstrated by the two maturity categories enable for a easing in the intraspecific competition that might be experienced by such a generational population of fish in a spacially limited habitat as demonstrated for the Insuyu Stream.

The dietary composition of *Gobio insuyanus* offers a fascinating glimpse into the adaptability of aquatic species across different habitats, seasons, and maturity stages. Such dietary flexibility can be attributed to the principles of optimal foraging, as discussed by Pyke *et al.* [51]. According to their theory, animals, including fish, tend to maximize their net energy intake per unit of foraging time. This could explain the observed preference for certain prey items in different habitats and seasons, as *Gobio insuyanus* might be selecting food sources that offer the highest energy return in a given environment. This observation is often demonstrated in organisms that are specialised feeders, or have a thin energy return margin, such as selective or non-selective top down benthic feeders, often exhibited in differing species of marine tusk shells [52].

The food items recorded in this study for all *G. insuyanus* individuals analysed demonstrate a preference for benthic inhabiting prey. This is evident not only in their downward facing mouth structure, but also backed up by the large amount of benthic sourced detritus found in the stomachs of all sampled individuals. This highlights the importance of the benthic make-up of the Insuyu Stream, and how critical it is as the main food source for *G.insuyanus*. Agricultural activities that might damage the benthos directly such as sourcing of riverbed sediment, and indirectly such as euthrophication or water loss needs to be fully controlled and prevented if this critically endangered species can survive the coming future.

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