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# Fish Biozonation in the Balkan Peninsula, Especially in Bulgaria: A Challenge

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**Abstract:** Fish are important elements of aquatic ecosystems. Their communities naturally follow the river continuum and have been well described in the western European freshwater watersheds. In regions of higher endemism, such as the Balkan Peninsula, the widely accepted fish zonation of Illies is doubtfully relevant. In this study, a more suitable categorization of lotic and lentic freshwater ecosystems in Bulgaria is proposed, based on 389 multimethodological and multihabitat fish sampling occasions. Referent-type communities have been determined for 33 types of recognized water bodies. Adapted zonation of Illies is a rough estimation of the real distribution of fish assemblances in the country; moreover, sensitive and/or endemic species are generally of restricted distribution and abundance. In view of the lentic ecosystems, they can be divided into salmonid and cyprinid, at approximately 1500 m above sea level.

**Keywords:** fish communities; freshwater; river continuum; aquatic ecosystems

## 1. Introduction

EU member states are obliged to classify their surface water bodies on the basis of typology, which is relevant to natural species assemblage structure [1]. In view of describing the river continuum and as an important component of the aquatic biota, fish represent a target group for policy-relevant monitoring and water management [2]. As the Balkan Peninsula is a globally important biodiversity region [3], fish biozonation, as well as the determination of referent communities for each zone, have become more urgent. Efforts towards fish zonation have taken place in western Europe [4], or even wider [5], which are doubtfully applicable in southeastern Europe.

In Bulgaria, serious efforts have been made in order to describe the fish species in rivers or catchments, lakes, and dams [6]. Nevertheless, the officially accepted freshwater fish zonation includes only the salmonid and the cyprinid zones, without further clarification [7]. This is a very rough estimation due to the fact that native salmonids have been registered even at low-altitude sites (Veleka and Rezova R. drainages) [6,8]. A mixed cyprinid zone could potentially include Balkan barbel, as well as bream, which are species with different biology and habitat preferences. These available data are only qualitative without further quantitative expressions, which are crucial for the assessment of aquatic ecosystems.

From another point of view, the widely accepted zonation of Illies [9] is not relevant concerning the Balkan Peninsula. The relatively higher percentage of endemism complicates the attempt for biozonation, as different key species appear, e.g., Asia Minor interglacial colonizers (*Oxynoemacheilus bureschi* Drensky, 1928) [10], Ponto-Caspian relicts (gobiids; *Rutilus frisii* Nordmann 1840; *Petroleuciscus borysthenicus* Kessler 1859), and brackish species in estuaries and lagoons [6]. Moreover, a key and referent species accepted by Huet [11], the grayling *Thymallus thymallus* Linnaeus1758, is absent in rivers of southeastern Europe, especially Bulgaria. Another important issue to be taken into account is the natural lack of the intolerant *Alburnoides* sp. in the East Aegean Basin [6]. Moreover, the distribution

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of recently established alien species [12] could be predicted in potential habitats, in order to undertake adequate measures for their management.

At the moment, no freshwater fish biozonation based on quantitative data and connected to the typology of certain water bodies has been achieved. Concerning the current situation, the study aims to propose referent typology-connected fish communities, which reflect the river continuum, as well as the natural and artificial lentic water bodies in Bulgaria.

## 2. Materials and Methods

### *2.1. Study Area*

Bulgaria is localized in the southeastern part of Europe and is approximately in the middle of the Balkan Peninsula. The landscape is multifarious, characterized by various mountainous areas (e.g., Haemus, Pirin, Rila, Rhodope, etc.) and separated by lowland valleys crossed by wide rivers, such as the Danube and its main tributaries, the Maritsa/Evros R. and tributaries; Kamchia R., Struma R., and Mesta R. The country belongs to both the Pontic and the Aegean biogeographic regions [13], defined also as Ecoregions 12 and 7, accordingly, following the classification of the water framework directive [14]. A more thorough division, as officially accepted by the national river basin management plans 2016–2021 [15], better illustrates the main fish biogeographic regions as west and east Aegean, the Danube, and the Black Sea basin directories/watersheds (Figure 1).

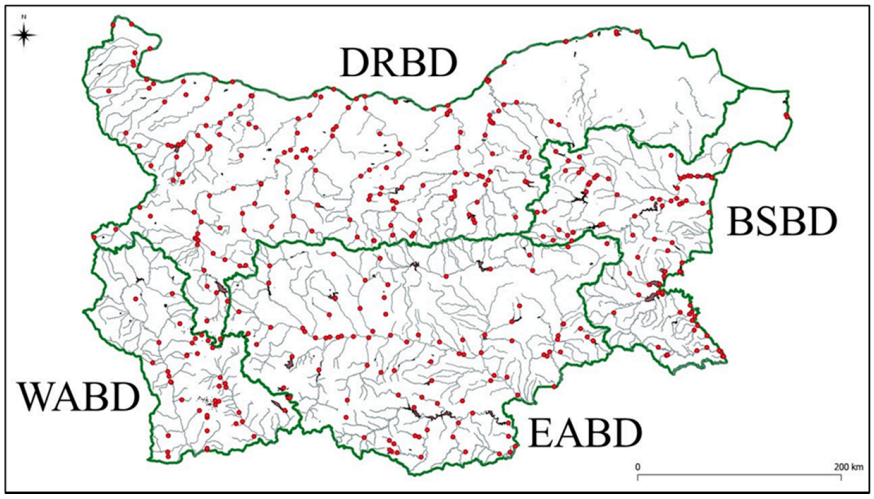


**Figure 1.** Sampling points for the determination of type-connected referent fish communities and biozonation in Bulgaria. (DRBD: Danube basin; BSBD: Black Sea basin; EABD: East Aegean basin; WABD: West Aegean basin).

### *2.2. Sampling and Data Analysis*

Fish sampling was performed during the period 2014–2020 in 389 sampling sites (Figure 1; Supplement S1a), which represent the typology of all national surface freshwater bodies according to Bulgarian legislation. Accordingly, they are divided into "rivers," "lakes," and "transitional waters," with 33 types in total, which are characterized by various hydromorphological parameters, including altitude [15].

A multihabitat sampling methodology was applied In view of the specific hydromorphological conditions of each sampling station: electrofishing [16], beach seine [17], gill nets [18], and underwater visual census [19]. When appropriate, more than one sampling method was used per station (e.g., in Danube R., lakes, and reservoirs). Every registered specimen above 2 cm in length (allowing a proper determination of species) was included in the dataset. In order to clarify the natural and specific fish communities, there were chosen sampling sites impacted by varying anthropogenic pressure for each type of water body in Bulgaria, according to available data [20] and field observations.

Moreover, concentrated historical data were used to precise the distribution of fish species in the Bulgarian rivers during the past [6,21]. Entirely extinct or doubtfully determined species have not been taken into account (e.g., *Acipenser nudiventris* Lovetsky 1828).

The number of sampling points per each type of water body is included in Table 1.

**Table 1.** Count of sampling points for the determination of type-connected referent fish. communities and biozonation in the Bulgarian freshwater bodies and basin directories/watersheds, according to the classification of Cheshmedjiev et al., 2010 [14].

| Water Body Type (L = lake) | Count | Water Body Type (R = river) | Count | Freshwater Basin | Count |
|---|---|---|---|---|---|
| L01 | 5 | R01 | 9 | Black Sea | 82 |
| L02 | 5 | R02 | 27 | Danube River | 173 |
| L03 | 6 | R03 | 14 | East Aegean | 93 |
| L04 | 4 | R04 | 58 | West Aegean | 41 |
| L05 | 3 | R05 | 12 | | |
| L06 | 3 | R06 | 15 | | |
| L07 | 3 | R07 | 32 | | |
| L08 | 5 | R08 | 35 | | |
| L09 | 8 | R09 | 7 | | |
| L10 | 4 | R10 | 9 | | |
| L11 | 5 | R11 | 9 | | |
| L12 | 5 | R12 | 13 | | |
| L13 | 5 | R13 | 12 | | |
| L14 | 4 | R14 | 40 | | |
| L15 | 5 | R15 | 8 | | |
| L16 | 5 | R16 | 9 | | |
| L17 | 5 | | | Total | 389 |

Raw data from electrofishing, beach seine and underwater visual census were unified and represented as ind./ha. Data from gill nets were calculated as ind./100 $m^2$ per hour CPUE. As most water bodies in Bulgaria are affected by various anthropogenic factors, the least-disturbed Bulgarian sites are characterized by near-reference conditions. According to the criteria concerning the common European river types [20], 28 referent sites have been recognized.

Reference communities were derived from a combination of:

- Best available (=near-reference) sites: Fish index values as calculated according to Belkinova et al. [22] preferably higher than 0.85 for the last sampling years.
- Historical data: species community, mainly presence/absence (partly dominance).

The heterogeneity of the data, the lack of reliable historical evidence (especially for non-fish species), and anthropogenic impacts on natural distribution patterns make it difficult to derive metric boundaries based on statistical analysis with sufficient confidence. Taxa were classified as dominant, accompanying, or rare species according to expert judgment, but based on field data and taking into account comparable methodological approaches in other EU member states. Based on the obtained field data from least-disturbed sites and following a comparable approach in the intercalibrated assessment method developed for Alpine rivers in Austria [23], the reference communities for each water body type were derived as follows:

- total referent abundance = 10,000 ind./ha as accepted by the national fish index
- relative proportion of dominant species >75%

- relative proportion accompanying species 20–75%
- relative proportion of rare species 5–20%

Taxa with a relative proportion below 5% were not included in the reference communities.

Afterward, sister and/or endemic species in the same genus were categorized together, as of the same guild, e.g., *Chondrostoma nasus* Linnaeus, 1758 with *Chondrostoma vardarense* Karaman, 1928, etc.

Field spatial data concerning fish presence and abundance were matched to the typology of the Bulgarian freshwater bodies. In order to obtain more precise categorization, a PCA analysis was undertaken, following the above assumptions.

The determination of fish coenobitic zones of Illies [9], was based on the results of the PCA analysis. A species/species complex was assumed as a referent for the characterization of a fish zone if dominant in all recognized Bulgarian river types included in this zone.

A similar approach as above was followed in terms of the lentic water bodies to define referent fish communities, and altitude was used as a numerical parameter without further categorization.

Alien species were not included in the whole dataset.

## 3. Results

On the basis of multihabitat and multimethodological fish sampling in 389 Bulgarian freshwater bodies, 157,320 fish specimens were established belonging to 84 species (Supplement S1b). In view of the available historical data and the performed sampling, a total of 107 relevant species have been recognized in order to derive the referent communities. A total of 105 species (93 native) could potentially be found in lotic ecosystems and 41 species/taxa (34 native) in lentic due to standardized fishing efforts. 38 are common to both ecosystem types, and 68 are registered only to rivers. One inhabits only brackish L09 lakes (*Proterorhinus marmoratus* Pallas, 1814), and the euryhaline *Knipowitschia longecaudata* Kessler 1877 is established only in some L07 and L08 lakes.

Due to the heterogeneity of fish fauna in some river types, they have been further divided: R11 in small and big (according to their catchment area and length), R14 is a, b, and c subtypes (according to Cheshmedjiev et al., 2010 [14]) (supplement S1c), and R09 is excluded from the adapted classification of Illies [9], as presented in Figure 2 and Table 2 since only *Gobio obtusirostris* Valenciennes 1842 is the only natural inhabitant. Type R11 was excluded also, due to the specific fauna and its division into small and big.

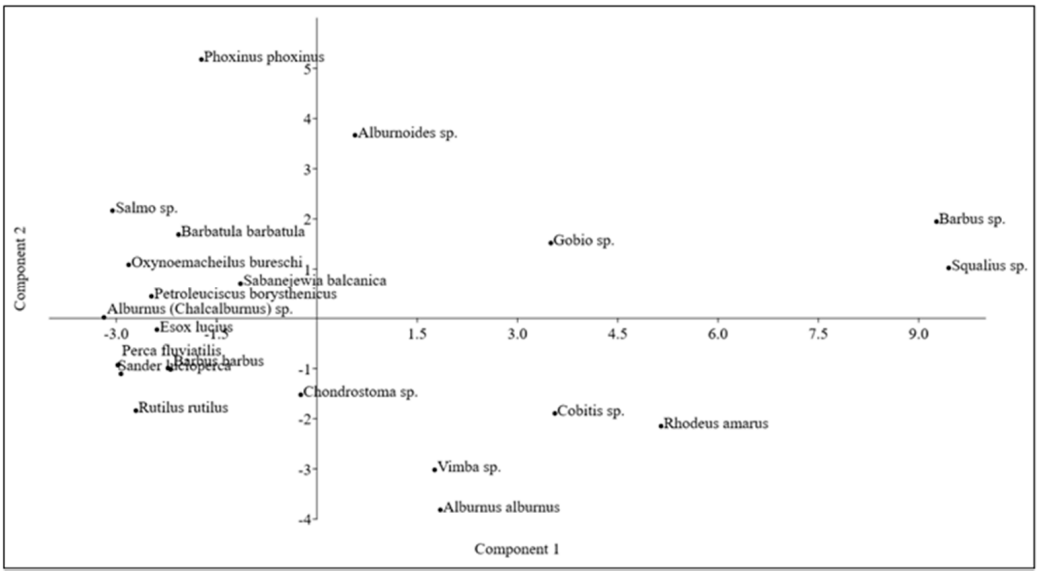


**Figure 2.** PCA scatterplot of Bulgarian fish species inhabiting Bulgarian rivers with an occurrence of more than 10% in each river type.

**Table 2.** Fish zonation according to Illies 1961 based on the established occurrence of freshwater fish species in Bulgaria.

| Zone | Species | | | | | |
|---|---|---|---|---|---|---|
| epirythral | *Salmo* sp. | *Phoxinus* sp. | | | | |
| metarhythral | *Barbus* sp. | | | | | |
| hyporhythral | *Barbus* sp. | *Squalius* sp. | | | | |
| metapotamal | *Barbus* sp. | *Squalius* sp. | *Rhodeus amarus* | *Cobitis* sp. | *Vimba* sp. | *Alburnus* sp. |
| epipotamal | *Barbus* sp. | *Squalius* sp. | *Rhodeus amarus* | *Cobitis* sp. | | |
| Transitional | all brackish *Alburnus* sp. (without *A. alburnus*) | *Petroleuciscus borysthenicus* | | | | |

The performed PCA analysis illustrates a two-way separation of the established species, in accordance with their occurrence and habitat preference (Figure 2). Along the X-axis, the occurrence of a species in all basins/types/zones increases (variance = 55.164). The Y-axis indicates the increase in rheophilic species on account of less rheophilic species (variance = 16.895). The *Alburnoides* sp. complex is also comparatively close to this group despite the fact that it does not naturally inhabit the East Aegean Basin.

Based on the above assumptions, the most frequent species were categorized according to the zonation of Illies [9], except for native salmonids, as well as *Phoxinus* sp. These are the single inhabitants of the metarhythral, often with low abundance or even absence (Table 2). This is expected as a solid number of the metarhythral streams in the country are not naturally inhabited by fish, due to the essential water flow fluctuations, especially during the dry season [24].

In view of the investigated lentic water bodies, referent communities have been defined according to their typology (Supplement S1d), and they can be grouped alternatively into two main categories:

1. Low-altitude lakes, where cyprinid fish communities together with percid predators, as well as *Silurus glanis* Linnaeus 1758, are common.
2. High-altitude lakes, which are inhabited by primarily salmonid fish communities.

These two categories are delimited at approximately 1500 m above sea level as derived by sampling data.

## 4. Discussion

This study has proposed two classifications for characterizing the fish communities of the Bulgarian lotic water bodies: one is detailed (Supplement S1c) and the other is briefer and according to Illies [9] (Table 2). Several arguments appear to accept or reject each. The brief approach is probably more user-friendly, especially when used by non-specialists, such as various stakeholders. It also follows a common European strategy. Nevertheless, certain important information is missing by generalization, e.g., the distribution of both *Chondrostoma* sp., two sensitive and potamodromous species inhabiting from metarhythral to epipotamal, but natively absent in the Black Sea basin. *Alburnoides* sp. is another important complex of sensitive species from metarhythral to epipotamal, but is not found in the East Aegean Basin.

As altitude decreases to lowlands, the precision also decreases. Meta- and epipotamal should include predators such as *Perca fluviatilis* Linnaeus 1758, *Esox Lucius* Linnaeus 1758, *Sander lucioperca* Linnaeus 1758, and *Silurus glanis*, as well as *Rutilus rutilus* Linnaeus 1758 and *Cyprinus carpio* Linnaeus 1758. This is relevant concerning the Danube and East Aegean Basins, but not the West Aegean Basin, especially concerning the R13 type (Supplement S1c). Another significant species common to the lowland rivers is the bream *Abramis brama* Linnaeus, 1758, which is considered native only to the Danube basin.

From the available data, it can be concluded, that due to the higher level of endemism (four watersheds with specific ichthyogeography each), common species and/or complexes of sister species are rare. Relatively tolerant taxa predominate in most of the widely accepted fish zones of Illies [9].

The detailed classification is thorough and can be used as a reference for further assessment, as well as for accurate stakeholder decisions, e.g., the determination of the ecological status of aquatic ecosystems according to the national legislation, in particular, Ordinance H4 of 2012 and amendments [25]. As a basis, it can be valuable for modeling NATURA 2000 and/or other endangered species' distribution [26]. HPP's management is also a crucial issue [27]. Without a detailed fish zonation, adequate fish passes cannot be predicted. At the moment, the national legislation does not recognize differentiation of fish passes according to fish zonation, e.g., for salmonids, rheophilic migratory cyprinids, other cyprinids such as *Rutilus rutilus*, etc. Ecological requirements of local fish faunas should thus determine the hydrological parameters of each fish pass.

This classification also becomes a trigger for a wider effort to determine fish communities and their zonation in the region. Recently, a valuable Pan-European classification of lampreys and fishes has been created according to their longitudinal and lateral distribution traits [5]. Nevertheless, endemic and in most cases sensitive species are lacking due to the wider range of the survey. On the opposite hypothesis, a disadvantage of zonation concepts is that fish zones could be defined by local indicator species, which limits their widespread application [4]. According to our data, only epi- and metapotamal fish fauna from the Danube basin could be included properly in the fish zonation of Illies [9]. The Black Sea, East, and West Aegean basins are characterized by specific fish faunas, which do not match this model.

In the area, only single surveys have been achieved concerning small catchments [28], but not entire basins.

Based on these assumptions, it is appropriate to accept the detailed reference communities connected with typology and reconsider the more general and unsuitable zonation of Illies in Bulgaria. The proposed classification, at first sight, is simply determined. On the other hand, further modeling is unsuitable due to a hydromorphological heterogeneity, reflected in the higher level of endemism in the area [3].

Nevertheless, an even more thorough revision must be undertaken to clear some typology mismatches established by sampling, e.g., in the R5 type, rivers from 3–4 to more than 50 m in width have been included, supporting distant fish communities. Type R13 in the West Aegean Basin is also doubtfully characterized as epipotamal because it supports poor rheophilic and relatively tolerant fish fauna (Supplement S1c). At this stage, it is difficult to use only fish for further clarification, since macrozoobenthos and hydromorphological characteristics are also crucial for the precise identification of river typology [14].

Concerning lentic water bodies, the zonation of Illies [9] is not relevant by definition, as initially proposed for rivers. Moreover, different lentic types in Bulgaria can be localized at the same altitude zone (Supplement S1d). It is widely known that reservoirs are accepted as heavily modified water bodies (HMWBs) or modified rivers, and their fish fauna is accordingly altered if compared with that of the adjacent river segments [29]. In parallel, a solid number of reservoirs are used for aquaculture in their entire volume [30], which determines their fish fauna as case-sensitive.

The knowledge of species-specific fish biology, ecology, habitat use, and placement within aquatic food webs is critical for science-based conservation and management [31]. In particular, classifications of fish communities are critically important in biodiversity conservation, since individual fish assemblages or community units may warrant conservation actions [32]. Freshwater conservation actions are based on the distribution of freshwater species, their patterns of endemism, and the exact threats imposed on certain species in certain habitats, especially in the area of the Balkans [13]. If these assumptions are lacking, management and conservation actions are of little effect. Based on the proposed

type-specific classification in this research, several similar issues concerning the fish fauna in the Bulgarian and adjacent watersheds can be solved.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8020091/s1, Spreadsheet S1a: fish sampling points for the determination of the referent communities in the Bulgarian surface freshwater bodies; Spreadsheet S1b: established fish species by sampling in the Bulgarian surface freshwater bodies; Spreadsheet S1c: referent fish communities in the Bulgarian lotic freshwater bodies; Spreadsheet S1d: referent fish communities in the Bulgarian lentic freshwater bodies.

**Author Contributions:** A.A.: sampling, data acquisition, manuscript preparation; L.P.: sampling, manuscript preparation; M.S.: sampling, manuscript review; H.Z.: sampling, manuscript review; G.W.: data acquisition and analysis, manuscript review. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** Not applicable: all sampled fish were released alive immediately after their identification in situ. Sampling was performed under local and EU legislation.

**Data Availability Statement:** The data presented in this study are available in the article and the Supplementary Material. Additional data are available on reasonable request from the MOEW-Bulgaria.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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