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***Comparative analysis of the trophic spectrum of fish
populations in the Neajlov River basin***

PHD THESIS ABSTRACT

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Abstract

The topic of this paper was chosen due to the need to better understand the trophic relationships of fish communities in freshwater ecosystems in Romania, in the context of recent influences of microplastic pollution. The Neajlov River basin offers an ideal setting for such research. The choice of this topic was motivated by the lack of local integrated studies that correlate the structure of the fish diet with the presence of microplastics and by the need for a reference assessment that can be used in future research.

The paper makes an original contribution by simultaneously analyzing the trophic spectrum and microplastic contamination at the species level within a geographical area that is poorly documented from an ecotoxicological perspective. Although the samples were collected in 2006, the results can be used as a reference standard for current and future comparative studies.

The research hypothesis assumes that the structure of the diet of fish in the Neajlov River basin reflects both the food preferences of the species and the availability of trophic resources, and the ingestion of microplastics is influenced by the size of the fish, not by trophic diversity.

The purpose of this work is to evaluate the trophic structure and the degree of microplastic contamination in fish communities of the Neajlov River basin, to understand how fish utilize trophic resources.

Objective 1: Identify the trophic spectra of fish species in the Neajlov River by analyzing stomach contents and determining the taxonomic groups consumed.

Objective 2: Evaluate the diversity and distribution of consumed trophic resources.

Objective 3: Determine the degree of trophic overlap between species to estimate the intensity of interspecific competition.

Objective 4: Investigate the presence and distribution of microplastics in the stomachs of fish and evaluate the types of particles ingested.

Objective 5: Correlating microplastic contamination with ecological factors, such as body weight or trophic diversity.

The aim is to outline a detailed profile of the trophic relationships between fish species in the Neajlov basin, with a focus on overlaps between trophic niches, and to identify patterns of microplastic contamination correlated with species and feeding habits. At the same time, the absence of a direct correlation between trophic diversity and the level of microplastic contamination is anticipated. Still, the involvement of relevant morphometric factors in explaining the ingestion of plastic particles is estimated.

Chapter 1 briefly presents the importance of trophic spectrum analysis in the study of aquatic ecosystems, highlighting the feeding relationships between fish species and other organisms, as well as the impact of invasive species on native ones. The main methods of diet analysis are described, such as examination of stomach contents. A systematic classification of fish species from the Neajlov River basin is carried out, with details about the habitat, trophic regime, and ecological behavior for each species. In the second part of the chapter, microplastics are defined and their sources are presented, as well as the harmful effects on aquatic ecosystems. The vulnerability of ichthyological fauna to contamination with microplastics, which can be ingested directly or indirectly, affecting the health of aquatic organisms and, implicitly, the ecological balance, is emphasized.

Chapter 2 presents details about the study area and the methodology used for sample collection and analysis. The study area is represented by the Neajlov River and its tributaries, with a watershed of approximately 3,795 km² and a total river length of 187.5 km. Six collection points were selected for harvesting fish species, located on the Neajlov River and its tributaries. The method used for capturing fish

was electrofishing, a non-invasive technique that allows collecting specimens without causing damage to aquatic fauna. The samples were transported to the laboratory, where they were analyzed by dissection and sorting, and the contents of the fish digestive tract were examined using a stereomicroscope. Ecological indices used for the analysis of fish diet include frequency of occurrence, relative abundance, and synthetic indices such as Shannon-Wiener, Pielou, and Pianka, which were calculated to assess the diversity and trophic competition within the studied ecosystem. To assess the impact of microplastics, stomach content digestion methods and visual analysis of the resulting samples were used, and the amount and types of microplastics were identified by microscopy. Thus, this chapter details the samples collection methods and technologies used for the analysis of faunal biodiversity in the studied areas, providing a starting point for further research.

In the study on the trophic spectrum of fish in the Neajlov River basin presented in Chapter 3, 8 fish species and 20 distinct taxonomic groups of macroinvertebrates were identified, with Chironomidae being predominant in the fish diet. The analysis showed a diet dominated by Chironomidae (73%), followed by Coleoptera (16.84%) and Diptera (11.79%), and trophic diversity, measured by the Shannon index, was moderate (0.931), but with an unequal distribution of resources, indicated by a low equity index (0.311). The study also revealed a high trophic overlap between species, such as *Gobio obtusirostris* Valenciennes, 1842, *Pseudorasbora parva* (Temminck & Schlegel, 1846), and *Alburnus alburnus* (Linnaeus, 1758), suggesting high interspecific competition, while other species, such as *Carassius gibelio* (Bloch, 1782), had a more distinct diet. Also, the analysis of hierarchical histograms confirmed these results, grouping species with a high trophic overlap and highlighting the differentiation between others.

Regarding the diet, the species *Squalius cephalus* (Linnaeus, 1758) showed a high diversity of prey consumed, with a Shannon index of 1.49, indicating a

diversified diet, but with an uneven distribution of prey, which suggests an opportunistic feeding. Regarding other species, *Pseudorasbora parva* and *Alburnus alburnus* were observed with very similar diets, based on Chironomidae larvae and other benthic prey. The same food was also consumed by *Lepomis gibbosus* (Linnaeus, 1758).

In conclusion, the analysis of the trophic spectrum of fish in the Neajlov River basin highlights both the significant overlap of trophic niches between many species, favoring competition for resources, and a trophic segregation in the case of other species, which adopt different feeding strategies to avoid direct competition. This diversity in trophic strategies contributes to the stability of the ecosystem and the coexistence of species in the analyzed habitat.

The study, presented in Chapter 4, investigates the presence of microplastics in the stomach contents of 200 individuals belonging to six fish species collected from various sectors of the Neajlov River, including the Milcovăț and Dâmbovnic tributaries. In total, 1831 plastic particles were identified, most of which were fibers ($\approx 87\%$), followed by films ($\approx 10\%$) and fragments ($\approx 3\%$). Particle sizes were predominantly below 5 mm (microplastics), and the most frequently encountered colors were blue, colorless, and red. The highest average microplastic load was recorded in the species *Babka gymnotrachelus* (Kessler, 1857) in the Milcovăț area (16.08 particles/individual), and the lowest in *Squalius cephalus* from station 1 (Neajlov) (5.2 particles/individual). Multiple regression analysis indicated a significant correlation between body weight and the amount of microplastics ingested for some species, but not for others. No significant correlation was observed between the trophic diversity of fish and the degree of microplastic contamination (Spearman $\rho = -0.41$; $p = 0.241$). The results highlight the widespread presence of microplastics in the Neajlov River ecosystem and emphasize the importance of considering ecological and morphometric factors in assessing contamination risk.