Summary

Amphibians are vital components of global biodiversity and fulfill diverse ecological roles, including controlling insect population, being part of the food chain themselves and serving as environmental health indicators. Therefore, it is very concerning that amphibian populations are in rapid decline worldwide, which is generally attributed to factors such as habitat loss, environmental pollution, climate change and emerging diseases. Concurrently, the issue of pervasive microplastic pollution has gained prominence due to its potential to affect aquatic ecosystems in many not yet understood ways. Microplastics, miniature plastic particles, originating from a plethora of sources, understandably exhibit various shapes, colors, sizes, and chemical compositions. When present in aquatic environments, for example suspended in the water column or as a component of sediments, microplastics pose risks to aquatic biota. Amphibians, particularly their vulnerable larval stages, are especially susceptible. The impact of environmental microplastic pollution is multi-layered. On a molecular level, effects like desorption of toxins and disruption of physiology are observed. At the individual level, after ingestion of microplastics, changes in behavior, morphology, or the immune system may occur. Finally, at the ecosystem level, microplastic pollution can lead to disruption of habitat structure or biogeochemical processes.

This PhD dissertation focuses on microplastic pollution of amphibian habitats located within the Lubusz Voivodeship. Microplastics were extracted from bottom sediments, surface water, and dead (desiccated) amphibian larvae collected in 23 sampling sites. These sites were all small inland water bodies, including ponds, puddles, and ditches. The sites experienced various levels of human impact (anthropopressure). In total, 934 amphibian larvae belonging to ten different species were collected and analyzed. These were: *Bombina bombina* (Bombinatoridae), three newt (Salamandridae) species, three toad (Bufonidae) species, three frog (Ranidae) species – including water frogs (the *Pelophylax esculentus* complex, grouped together). In order to extract possible microplastics, each specimen was individually digested with H_2O_2. To measure the human impact (anthropopressure) at each sampling site, the proportion of artificially modified land cover within a one-kilometer radius buffer was calculated. This category of land cover included urban fabric, industrial and commercial units, mines, construction sites, and artificial non-agricultural vegetated areas. Moreover, in some statistical analyses, the share of agricultural land cover within the buffers was also considered. Both types of spatial analyses were conducted with a Geographic Information System.

Microplastic analyses involved determining their shape (morphological type), color, measuring their length in the longest diameter, their surface area, and analyzing their chemical composition. The latter encompassed a representative subset of the particles and employed ATR-FTIR spectroscopy. The spectra obtained from the samples were compared against reference spectra from spectral libraries. In order to calculate the diversity of shapes of bioaccumulated microplastics, Levin's index was used. Statistical analyses included several different methods. These encompassed principal component analysis, a mixed-effects negative binomial regression model, a chi-square goodness-of-fit test, post-hoc tests, ANOSIM and multipattern analysis (Indicator Species Analysis).

The study found that microplastic pollution is prevalent among amphibian larvae (found in 73% of specimens). Of the studied amphibian families, toad (Bufonidae) tadpoles were the most polluted. Importantly, carnivorous amphibian larvae (Caudata larvae) were significantly less polluted than omnivorous amphibian larvae (Anura larvae), even if they inhabited the same water body. Both the number of microplastic particles and the diversity of their shapes in amphibian larvae were positively correlated with anthropopressure of a sampling site. To the best of my knowledge, this is the first study to prove that pattern. The diversity of microplastics' shapes was also influenced by species identity.

Among the microplastics extracted from amphibian larvae, fiber was the most common shape, blue was the most common color, while polyethylene turned out to be the most often identified synthetic polymer making up the particles. Fibers were found to be on average longer than any other shape of particle distinguished in the study, which means that they were longer than flakes, fragments and granules. Granules had the biggest surface area. Moreover, a statistically significant difference in the surface area of microplastics was noted between the different amphibian families. Another interesting pattern discovered in the course of this study was a positive correlation between the size of amphibian larvae, and both the length and the surface area of flake-shaped and granule-shaped microplastics. On the other hand, a negative correlation was found between anthropopressure and the same microplastics' characteristics.

The results of the analyses of microplastics extracted from surface water samples showed that the most often identified shape, color, and polymer were fragment, white, and polypropylene, respectively. For the particles extracted from bottom sediment samples, it was fiber, blue and poly(ethylene-propylene), respectively.

Within-site similarities in shape, color, and polymer type of microplastics extracted from amphibian larvae, bottom sediments, and surface water were evaluated. Analyses revealed statistically significant similarities in shapes and colors between microplastics from water and those from amphibian larvae. Similar congruence, although less pronounced, was also observed between microplastics extracted from bottom sediments and amphibian larvae. However, the chemical composition (polymer type) of microplastics from water, sediment, and amphibian larvae did not exhibit any similarities beyond what would be expected by chance.

Results of the study, by clarifying intricate relationships between anthropopressure and microplastic pollution, significantly contribute to our knowledge of the ecotoxicology of small inland water bodies. The observed within-site similarities of microplastic profiles of those extracted from water and amphibian larvae suggest that these organisms primarily ingest microplastics from water rather than from bottom sediments. All these findings emphasize the interconnectedness within small inland water bodies and highlight the need to develop comprehensive strategies for preventing and mitigating microplastic pollution in the habitats of protected species. Lastly, these findings highlight the urgent need for additional research to fully understand the long-term impacts of these pollutants on freshwater ecosystems and biology of protected species.