Chapter 5

Aquatic biodiversity along the Danube River

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Introduction

Biological diversity, or biodiversity, is defined as the variability among living organisms sourced from various environments, including terrestrial, marine, and other aquatic ecosystems, along with the interconnected ecological systems in which they exist (IUCN, 2000). It encompasses variation at the genetic (genetic variability), species (species diversity), and ecosystem (ecosystem diversity) levels. It is widely acknowledged that greater biodiversity in ecosystems, species, and individuals leads to greater overall ecological stability and resilience to disturbances including climate change. Since biodiversity includes entities with varying degrees of complexity and different temporal and spatial scales, it has a hierarchical structure (Cogălniceanu, 2007). Besides living beings (i.e., the biological component), it includes biotopes (i.e., the nonliving space occupied by them) that are also subject to changes in time and space. The assessment of biodiversity is complex and has qualitative and quantitative aspects, as it cannot be regarded as the sum of all the differences across genes, species, habitats, and ecosystems but rather as a measure of the variety of these differences.

Aquatic ecosystems and their floodplains, that is, the associated ecotonal areas are disturbance-dominated systems controlled by periodic floods that maintain and generate a high habitat diversity. Thus, the Danube River and its floodplains form a dynamic system, linked by the strong interactions between hydrological and ecological processes as described in the "flood-pulse concept" (Junk et al., 1989; Tockner et al., 2000). The Danube River has an extensive floodplain area covering over 17 million ha (Gren et al., 1995). Floodplains provide a variety of ecosystem services, ranging from water purification, habitat for biodiversity, flood control (Schober et al., 2015), wind protection, supply of food (including fish and game), and raw materials, as well as areas for tourism and recreation (Gren et al., 1995). The total annual value of the existing Danube floodplains for 1994 was estimated at 374 euros/ha; thus, the total annual value of the entire area of Danube floodplains corresponds to 650 million euros per year. Approximately two-thirds of this value is obtained in Romania including the Danube Delta (Gren et al., 1995). Despite the enormous benefits of floodplains, it is estimated that two-thirds of the Delta's ecosystem services have declined from 1960 to 2010 (Gómez-Baggethun et al., 2019). While Chapter 15 treats the concept of ecosystem services, the crucial role of floodplains in nature conservation is further described in Chapter 16.

Ecoregions of the Danube River Basin and Black Sea Coast

According to traditional zoogeographic and recent phylogeographic studies, the DRB represents a hotspot for European freshwater biodiversity. Before and after the ice ages, the geographic characteristics of the basin made it a valuable corridor

The Danube River and The Western Black Sea Coast. https://doi.org/10.1016/B978-0-443-18686-8.00019-6

FIGURE 5.1 Ecoregions of the Danube River Basin. ***Map lines delineate study areas and do not necessarily depict accepted national boundaries***. *Courtesy of ICPDR (2021).*

for migration and recolonization: freshwater organisms moved toward the East between the Ponto-Caspian region to central Asia, and to the Alpine and Mediterranean regions to the West, while the Danube's mainstem remained unglaciated, creating a "refuge." With the receding of the ice sheets, the species extended their range from this refuge to the rest of Europe. The Danube Delta is also a meeting point of Palearctic and Mediterranean biogeographic zones with many wetland habitats and rich biodiversity (Sommerwerk et al., 2022). The Danube flows through four European biogeographical regions: Continental, Pannonian, Steppic, and Black Sea (EEA, 2017). Out of nine ecoregions covered by the DRB, the Danube River flows through five: Central Highlands, Hungarian Lowlands, Dinaric Western Balkan, the Carpathians, and Pontic province (Fig. 5.1, https://www.icpdr.org/main/sites/default/files/nodes/documents/drbmp_update_2021_final_map_02_-_ecoregions.pdf). Each represents an area with a distinct biodiversity and specific management requirements; therefore, they serve as an essential basis for the classification of biologically relevant surface water types. Unlike most other European rivers, the Danube is only moderately developed, especially downstream of Vienna (Austria). Almost one-third of its length downstream of the Iron Gates dam is free-flowing, thus providing favorable hydromorphological conditions to freshwater and riparian species (ICPDR, 2015).

Biodiversity of the Danube River

It is estimated that over 2000 plants and 5000 animal species live in or along the Danube River (https://danubeparks.org/the-danube). Description and data on its biodiversity are dispersed across centuries (the first proper studies of biodiversity started in the 18th century), countries, universities, institutions, agencies, and vast amounts of literature.

Since 2001, with the establishment of the Joint Danube Survey (JDS), under the lead of the International Commission for the Protection of the Danube River (ICPDR), a significant effort has been made to collect and describe the biodiversity of particular taxa on the river basin scale and to improve the validity and comparability of water quality data and ecological status of the Danube River and its main tributaries. The water quality of the Danube River and the Black Sea, including various pollutants and microbiological quality, is described in more detail within Chapter 4 of this book. So far, JDSs have been carried out in 2001 (JDS 1, Literáthy et al., 2002), 2007 (JDS2, Liška et al., 2008), 2013 (JDS 3, Liška et al., 2015), and 2019−20 (JDS4, Liška et al., 2021). A joint database for the results was created (https://www.data.danubesurvey.org) and published. Over 140 different parameters have been studied, including parts of biodiversity that are relevant for ecological status characterization according to the European Water Framework Directive (EU WFD, 2000): phytobenthos, macrozoobenthos, macrophytes, phytoplankton, and zooplankton. A fish survey along the whole length of the river was carried out since 2007 during JDS2, and riparian birds and invasive species were added during JDS3 in 2013.

Algae

Evaluating the biodiversity of algae and cyanobacteria in the Danube River and associated floodplains becomes complex when all habitat assemblages, such as phytoplankton, phytobenthos, and periphyton, are considered. Also, the spatial patchiness, that is, longitudinal and cross-sectional differences or changes along seasons or years, alters biodiversity. Biodiversity estimates for microorganisms can be further complicated given different survey methods, and taxonomic identification and resolution.

Early investigations in the Austrian reach (Brunnthaler, 1900; Schallgruber, 1944) indicated pennate diatoms as dominant. Centric diatoms gained increasing importance in the 1960s (Wawrik, 1962), with *Stephanodiscus hantzschii* as a type species during all seasons. Szemes (1967) compiled the first systematic listing of all Danube plants. Kusel-Fetzmann et al. (1998) updated and largely extended these records, which included 2616 taxa for the entire Danube covering phytoplankton, phytobenthos, and periphyton. The dominant algal groups here were mainly Chlorophyta, followed by Bacillariophyta, with 1027 and 735 taxa, respectively (Fig. 5.2A; lower-ranked phytoplankton taxa are shown in B, with their average contribution to total biovolume across the 74 mid-stream samples from JDS2 in 2007).

The phytoplankton of midstream samples of the Danube River (Fig. 5.3) is dominated by Bacillariophytes (65%), mainly represented by centric diatoms (Centrales 57%, dominant: *Cyclotella meneghiniana, Thalassiosira weissflogii, Stephanodiscus hantzschii* and *S. parvus, Skeletonema potamos*, *Aulacoseira granulata*), while Pennales are less common (8.5%). This confirms former findings that diatoms are of decisive importance among microscopic assemblages of primary producers in the Danube River. Also, during JDS4 (April to September 2019) phytoplankton was dominated by taxa of Bacillariophyta (249), followed by Chlorophyta (224), Cyanobacteria (77), Ochrophyta (46), Euglenozoa (35), Charophyta (23), Cryptophyta (17), Myzozoa (10), and Choanozoa (1) (Stanković et al., 2020). When identifying functional species, that is, algae combined by their similar morphological, physiological, and behavioral traits, such as functional trait of motile and nonmotile phytoplankton taxa (details see Weithoff, 2003), among these assemblages, 29 functional

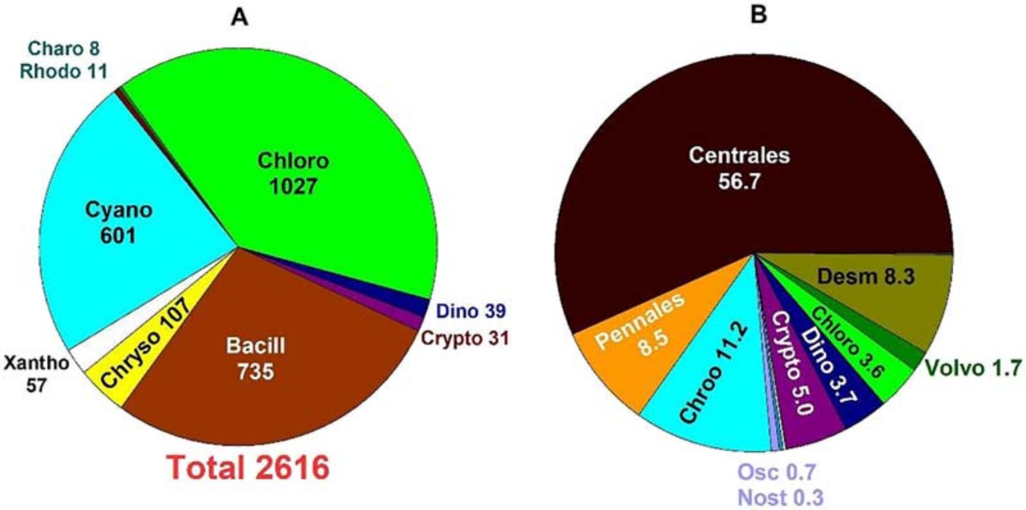

FIGURE 5.2 Phytoplankton taxa with their average biovolume contribution. Number of high (A) and lower-ranked (B) taxa averaged for the entire Danube, from the origin to the Black Sea. Cyano − Cyanobacteria in (A), with Chroo − Chroococcales, Osc − Oscillatoriales and Nost − Nostocales (in B); Bacill − Bacillariophyta (in A), with Centrales and Pennales (in B); Chloro − Chlorophyta (in A), with Chloro − Chlorococcales and Volvo − Volvocales (in B); Charo/Rhodo − Charophyta and Rhodophyta (in A), with Desm − Desmidiales of Charophyta (in B); Dino − Dinophyta (in A), with Dino − Dinophyceae (in B); Crypto − Cryptista (in A), with Crypto − Cryptophyceae (in B); Chryso − Heterokontophyta (formerly Chrysophyta, in A); Xantho − Xanthophytes (in A).

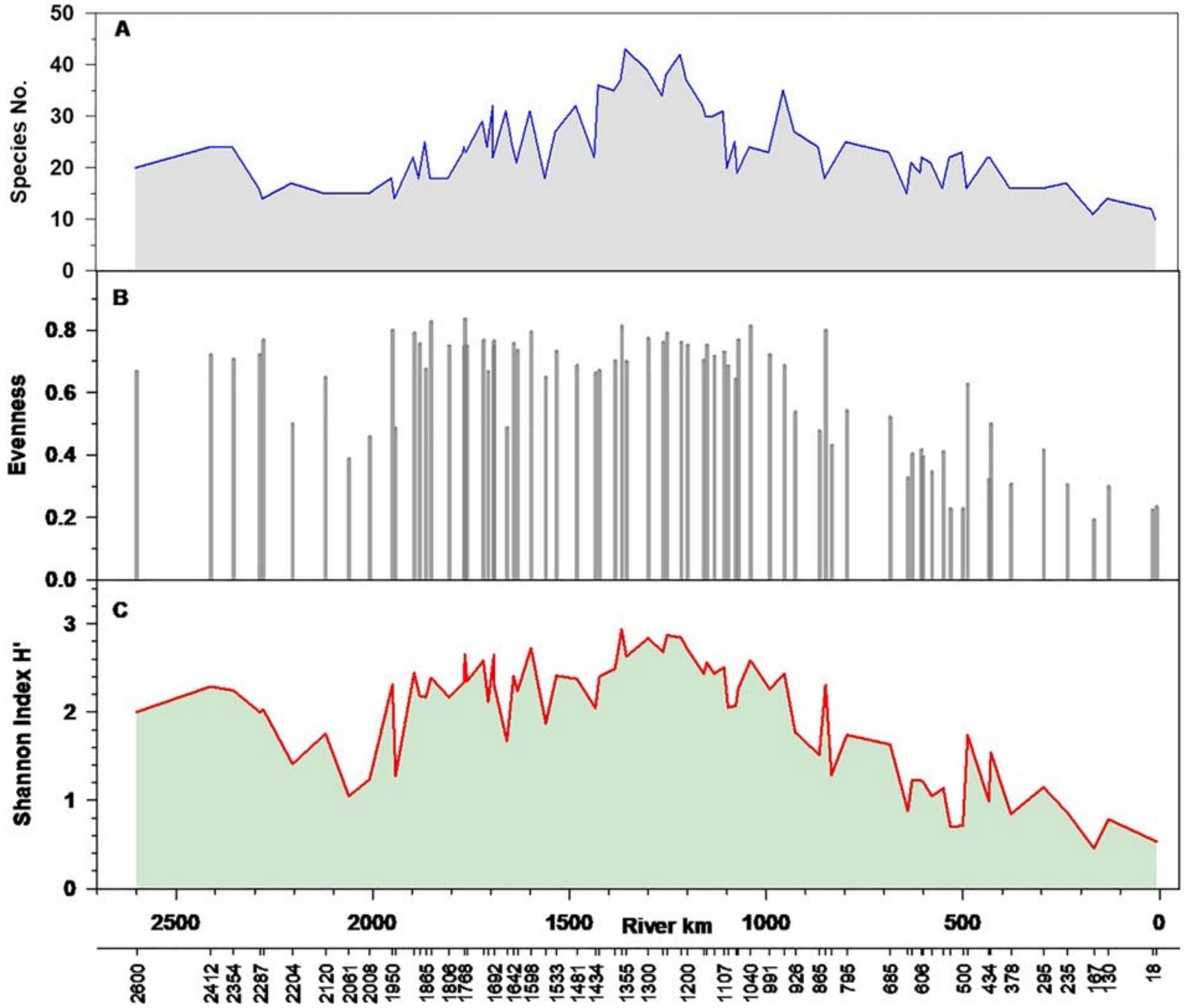

FIGURE 5.3 Longitudinal profile of phytoplankton species number, species evenness and Shannon diversity index.

phytoplankton groups were found. Abonyí et al. (2018) conclude that the change in functional diversity across sampling sites is a better proxy of human impacts than changes traced by single phytoplankton taxa development.

The spatial variability of river phytoplankton diversity along the Danube River from the city of Regensburg (rkm 2600) to the delta at the Black Sea (rkm 0) is shown in Fig. 5.3 (Dokulil and Kaiblinger, 2008). Concentrations of soluble reactive phosphorus (SRP) never exceeded 10 μg/L during the 6 weeks of the survey. Varying concentrations were largely related to the discharge but did not significantly change the number of species or diversity. Species richness and diversity indices peaked in the middle reach of the river (rkm 160−1200). Both indicators showed an evident spatial variability but seemed not to be significantly influenced by any inputs from tributaries. The decline of the Shannon diversity index (H′) in the lower stretch of the river (Fig. 5.3c) was paralleled by an almost equal decline in evenness (Fig. 5.3b). The decline in species number, evenness, and diversity (Fig. 5.3a−c) is likely caused by turbidity. The ups and downs of H′ were significantly related to species richness, explaining 50% of the variability (r' $= 0.50$, n $= 74$, F $= 73.2$, $P < .001$).

Further details of phytoplankton composition, seasonality, and long-term development in the Danube River can be found in Dokulil (2015). For more comprehensive and comparative data on general algal biodiversity, see Dokulil (2017).

In the temperate region, benthic algae (periphyton or phytobenthos) are the most successful primary producers in most of the streams, representing the main source of energy for higher trophic levels (Minshall, 1978; Lamberti, 1996). In large rivers, the leading role in primary production is governed by phytoplankton (Vannote et al., 1980), favored by the environmental conditions, which often restrict the development of algal biofilms to the littoral zone due to limited light availability and high flow turbidity. This is the reason why phytobenthos studies in large rivers such as the Danube are carried out in the bank areas, which provide suitable places for sample collection. Nevertheless, both phytoplankton and phytobenthos are valuable bioindicators of the environmental conditions: while phytoplankton mirrors the short-term changes, the attached benthic algae reflect the long-term dynamics of the aquatic ecosystem health (Hlúbiková et al., 2015).

A detailed historical overview of the Danube phytobenthos studies is given in Makovinská and Hlúbiková (2015). Since 2001 Joint Danube Surveys (JDS) have investigated more than 2500 km of the river Danube's longitudinal profile, resulting in the most comprehensive data along the whole river. The study of phytobenthos, one of the monitored aquatic communities, was focused primarily on the species composition, relative abundance, and bioindication (Makovinská et al., 2002, 2008; Hlúbiková et al., 2015; Fidlerová and Makovinská, 2021); however, in 2007 and 2013, also phytobenthos biomass measurements were included (Makovinská et al., 2008; Hlúbiková et al., 2015).

The phytobenthos assemblage has been investigated based on the diatom and nondiatom community in the first three JDS (2001, 2007, 2013), while in the year 2019, only diatoms were used. This resulted from the way the survey was organized, while the first three JDS had a common pattern (a Core Team of leading experts was responsible for sample collection, laboratory analysis, and evaluation), JDS4 was based on the activities of national teams (sampling, laboratory analysis) under coordination and advisory roles (including assessment of results) from the Core Team.

Benthic diatoms (Bacillariophyta) is the richest community. The number of taxa in the Danube varied in the range of 264−391 during all Joint Danube Surveys (Fig. 5.4). The distribution of the number of taxa at sampling sites along the Danube, common for three JDS, is given in Fig. 5.5. The differences among individual JDS in species diversity of diatoms were caused mainly by implementing progressive molecular biology methods in taxonomy and systematics (between 2001 and 2019) and by including national experts in JDS4. Integrating molecular methods allowed the division of the diatom community into several additional taxa, thus influencing the results of JDS3 and JDS4.

During JDS2, along the Danube and its main tributaries 200 diatom taxa occurred at more than one sampling site, 75 taxa recorded a frequency of over 20% and 13 taxa had a frequency of over 50% (Makovinská et al., 2008). By comparing

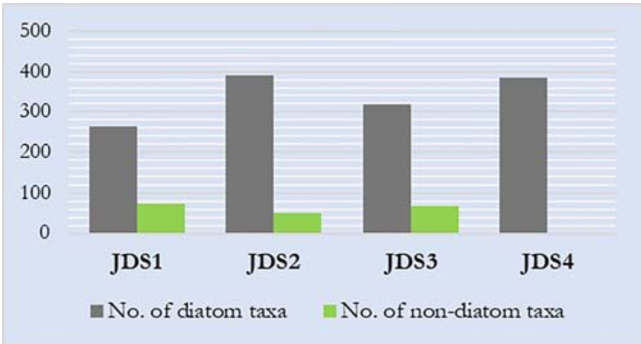

FIGURE 5.4 The number of taxa among benthic diatoms and nondiatoms during the Joint Danube Surveys.

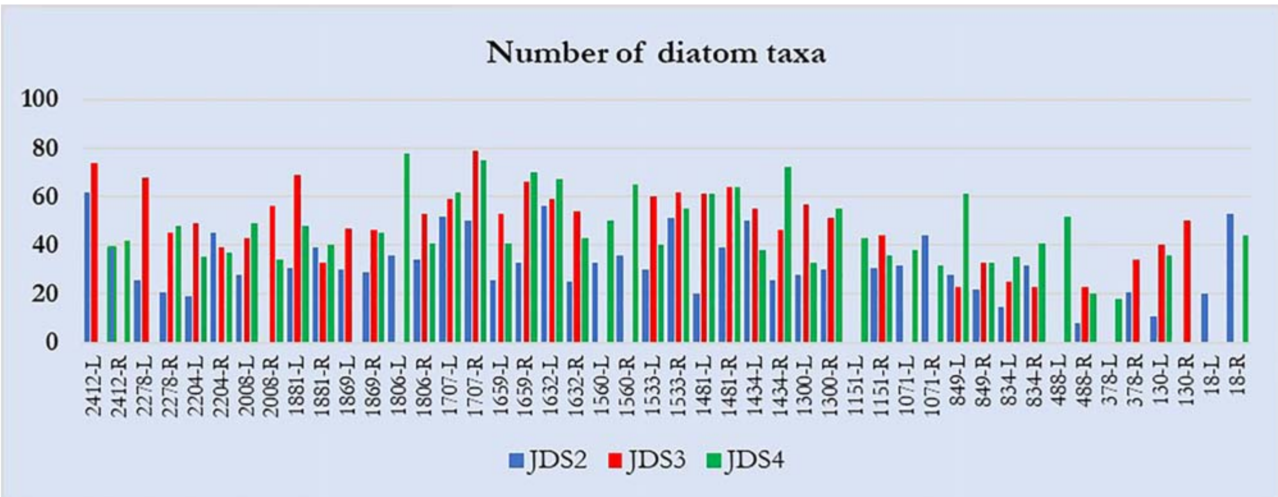

FIGURE 5.5 The distribution of the number of taxa at sampling sites along the Danube, common for three surveys. The x-axis states the respective river km and whether the sample was taken at the left or right bank.

the relative abundance of the dominant taxa, only 21 species obtained an average relative abundance higher than 1% at all sites, *Navicula recens* and *N. tripunctata* being the most abundant and frequent species. Among the most abundant and dominant genera recorded were, for example, *Amphora*, *Cocconeis*, *Eolimna*, *Gyrosigma*, *Luticola*, *Navicula*, *Nitzschia*, *Rhoicosphenia,* and *Reimeria.*

In total, 318 diatom taxa were found during JDS3, of which only 61 species reached a relative abundance of at least 5% (Hlúbiková et al., 2015). Concerning the species frequency, only 28 species occurred at most 50% of sites. The species occurring with a frequency of over 75% were, for example, *Amphora pediculus*, *Cocconeis placentula*, *Cyclotella meneghiniana*, *Navicula cryptotenella*, *N. recens*, *Nitzschia dissipata*, *N. fonticola*, *N. palea* var. *debilis,* and *N. palea*.

In JDS4, 385 diatom taxa belonging to 78 genera were identified in 72 samples (Fidlerová and Makovinská, 2021). 158 diatom taxa reached a relative abundance of over 1% in at least one sample. The most abundant and the most frequent species with a mean relative abundance of at least 5% and frequency of at least 10% of samples were *Achnanthidium delmontii*, *Amphora pediculus*, *Cocconeis euglypta*, *Cyclotella meneghiniana*, *Navicula recens*, *Nitzschia dissipata,* and *Skeletonema potamos*.

The diversity (Shannon H′) and evenness (J′) indices have been calculated for the sampling sites (both banks), which were investigated during JDS2, JDS3, and JDS4 (Figs. 5.6 and 5.7). The diversity index, which takes into account the proportion of each species in the studied ecosystem, ranged similarly in JDS2 and JDS3 (1.33−4.94; resp. 1.29−4.98), while in 2019 (JDS4), the interval was even wider (1.15−5.44) (Makovinská et al., 2008; Hlúbiková et al., 2015; Fidlerová and Makovinská, 2021). The equilibrium of the diatom community is expressed by the evenness (J′) index, which shows its wide range (0.28−1.0). The survey results in 2013 and 2019 pointed to a relatively stable diatom community balance in the upper and middle stretch of the Danube.

The nondiatom benthic community was investigated during JDS2 (2007) and JDS3 (2013) concerning species composition and biomass onboard and in the field in live samples.

In 2007, 52 taxa in total were identified at 124 sampling points along the Danube of the three main groups (Cyanobacteria, Chlorophyta, Rhodophyta) (Makovinská et al., 2008). Cyanobacteria were represented by filamentous species (*Heteroleibleinia fontana*, *H. kützingii*, *Homeothrix varians*, *Lyngbya martensiana*, *Oscillatoria limosa*, *Phormidium retzii*, *Ph. targestinum*), which occurred in more than 75% of samples. Coccal cyanobacteria were represented by the genera *Chroococcus*, *Chamaesiphon,* and *Pleurocapsa*. Green algae (e.g., *Cladophora glomerata*, *Hydrodictyon reticulatum*, *Spirogyra* sp*.*, *Stigeoclonium tenue*) were abundant in the Danube, mostly in shallow pools of the river. The red algae *Hildebrandia rivularis* and *Bangia artropurpurea* were found in the upper stretch of the Danube.

In 2013, 62 taxa in total were identified, and the three above-mentioned groups were confirmed (Hlúbiková et al., 2015). 40 taxa of cyanobacteria were represented mainly by genera such as *Calothrix, Heteroleibleinia*, *Homeothrix*, *Leptolyngbya*, *Lyngbya*, *Oscillatori*a, *Phormidium*, *Stigonema*, *Chroococcus, Chamaesiphon, Geitlerinema, Geitleribactron, Pleurocapsa, Stanieria*. As for the green algae, the same taxa as in 2007 occurred, however, *Pseudendoclonium basiliense* was found quite often down to the Delta. Red algae *Hildebrandia rivularis* and *Bangia artropurpurea* were confirmed in the Upper Danube, while *Thorea hispida* was found in the mouth of the Sava in Belgrade.

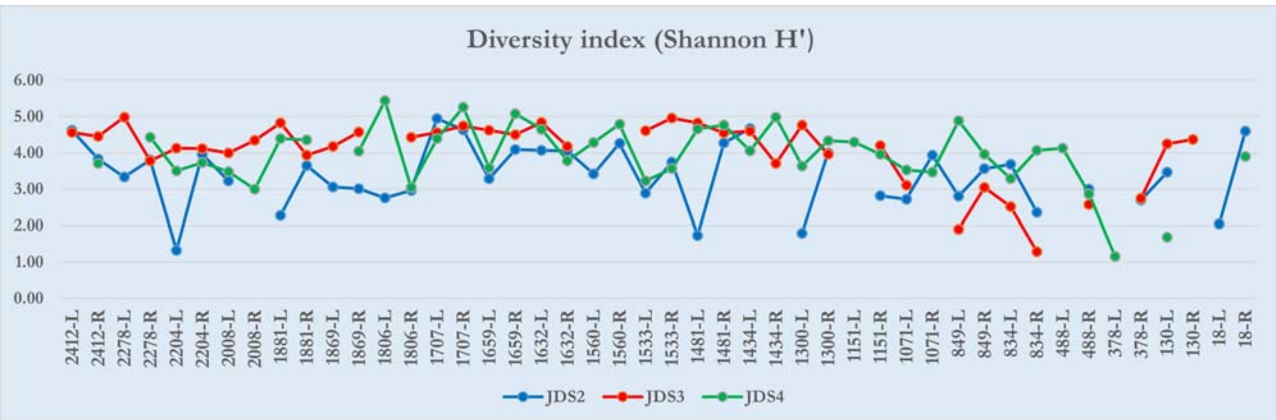

FIGURE 5.6 The diversity index (Shannon H′) at sampling sites along the Danube. The x-axis states the respective river km and whether the sample was taken at the left or right bank.

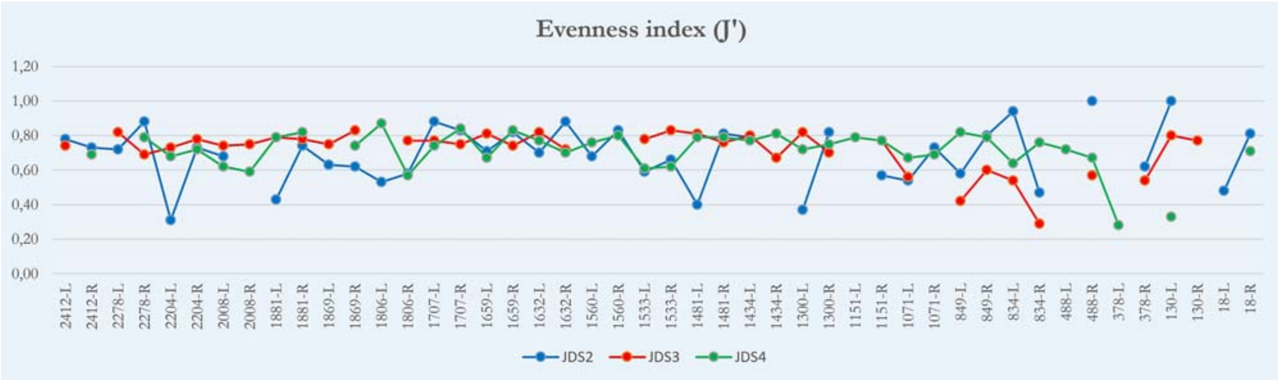

FIGURE 5.7 The evenness index (J′) at sampling sites along the Danube. The x-axis states the respective river km and whether the sample was taken at the left or right bank.

Macrophytes

In addition to water quality, the Danube River offers a wide range of abiotic habitat conditions, including varying water depths and flow velocities, substrate types, and water transparency. These factors serve as a basis for the development of highly diverse macrophyte vegetation. Results from detailed macrophyte mapping in the Danube are accessible for almost all riparian states, as, for example, for Germany (Pall and Janauer 1995, 2003; Pall et al., 2004; Schütz et al., 2004), Austria (Pall and Janauer 1998), Slovakia (Oťaheľová and Valachovič 2006; Oťaheľová et al., 2007), Hungary (Pall et al., 1996; Ráth et al., 2003; Janauer and Steták. 2003), Croatia (Ozimec et al., 2010), Serbia (Vukov et al., 2008), Bulgaria (Gyosheva et al., 2019), and Romania (Sârbu et al., 2011). However, all these studies only provide data from individual river stretches, which differ both in the year of mapping and in the level of detail regarding spatial resolution and taxonomy.

First overviews of the macrophyte flora in the more or less whole Danube Basin have been provided by Liepolt (1967) and Kusel-Fetzmann et al. (1998). From 2002 to 2004, a study covering almost the entire Danube River was done (Janauer et al., 2021). More recently, homogeneous macrophyte datasets covering the entire stretch of the Danube could be obtained during the Joint Danube Surveys 1 to 4, conducted in 2001, 2007, 2013, and 2019 (Literáthy et al., 2002; Liška et al., 2008, 2015, 2021). Throughout all JDS surveys, a total of 177 macrophyte species (71 hydrophytes, 66 amphiphytes, and 40 helophytes) were identified, including three charophytes, 47 bryophytes, four pteridophytes, and 123 spermatophytes. The stretches of the Lower Alpine Foothills Danube (rkm 2001 to 1807), the Pannonian plain Danube (rkm 1497 to 1045), and the Western Pontic Danube (rkm 943−375.5) were found to have a high number of species, while the Upper Course of the Danube (rkm 2786-2581), the Hungarian Danube Bend (rkm 1807-1497), and the Eastern Wallachian Danube (rkm 375.5−100) exhibited comparatively lower species diversity.

Fig. 5.8 shows the species diversity along the Danube River according to the results obtained during the JDS4 (Stanković and Bubikova 2020). The maximum number of macrophyte species found at one site was 37, located at the German-Austrian border. Here, the confluence of the river Inn leads to a clear dynamization of the flow regime. As a result, the species spectrum is enriched above all by the plant group of mosses, which is well adapted to such conditions. In general, inflowing rivers (e.g., the tributaries Morava, Sió, Tisza, Ialomita) seem to widen the range of species not only by influencing the hydromorphological conditions but also by bringing in some elements of their specific flora.

High species diversity is mostly, but not necessarily, associated with high plant quantities (Fig. 5.8). Macrophytes are typically found exclusively along the banks of the Danube River, extending to a water depth of approximately 1.5−2 m. Only in sections with slow currents and high water transparency, they may inhabit greater depths and, in some cases, occupy nearly the entire river width. Accordingly, lower plant masses were observed in the upper, more free-flowing, narrow course of the Danube, the Hungarian Danube Bend, and the Eastern Wallachian Danube. In contrast, the highest plant masses were observed in the impounded Danube section upstream of the Iron Gate (rkm 1075-943) and in the Danube Delta.

Approximately two-thirds of the total plant mass in the Danube is provided by hydrophytes, with amphiphytes contributing around 10% and helophytes around 20%. River stretches with near-natural banks have the highest helophytes plant mass, especially amphiphytes. Hydrophytes can attain high plant mass in various conditions, but characteristic changes in the compositions of growth forms are evident along the river course. The stretch through the Alpine foothills is dominated by mat-forming bryophytes, contributing more than 80% to the overall plant mass. As the river leaves the Alpine region, submersed rhizophytes become increasingly important, peaking in their proportion of the overall plant mass (about 70%)

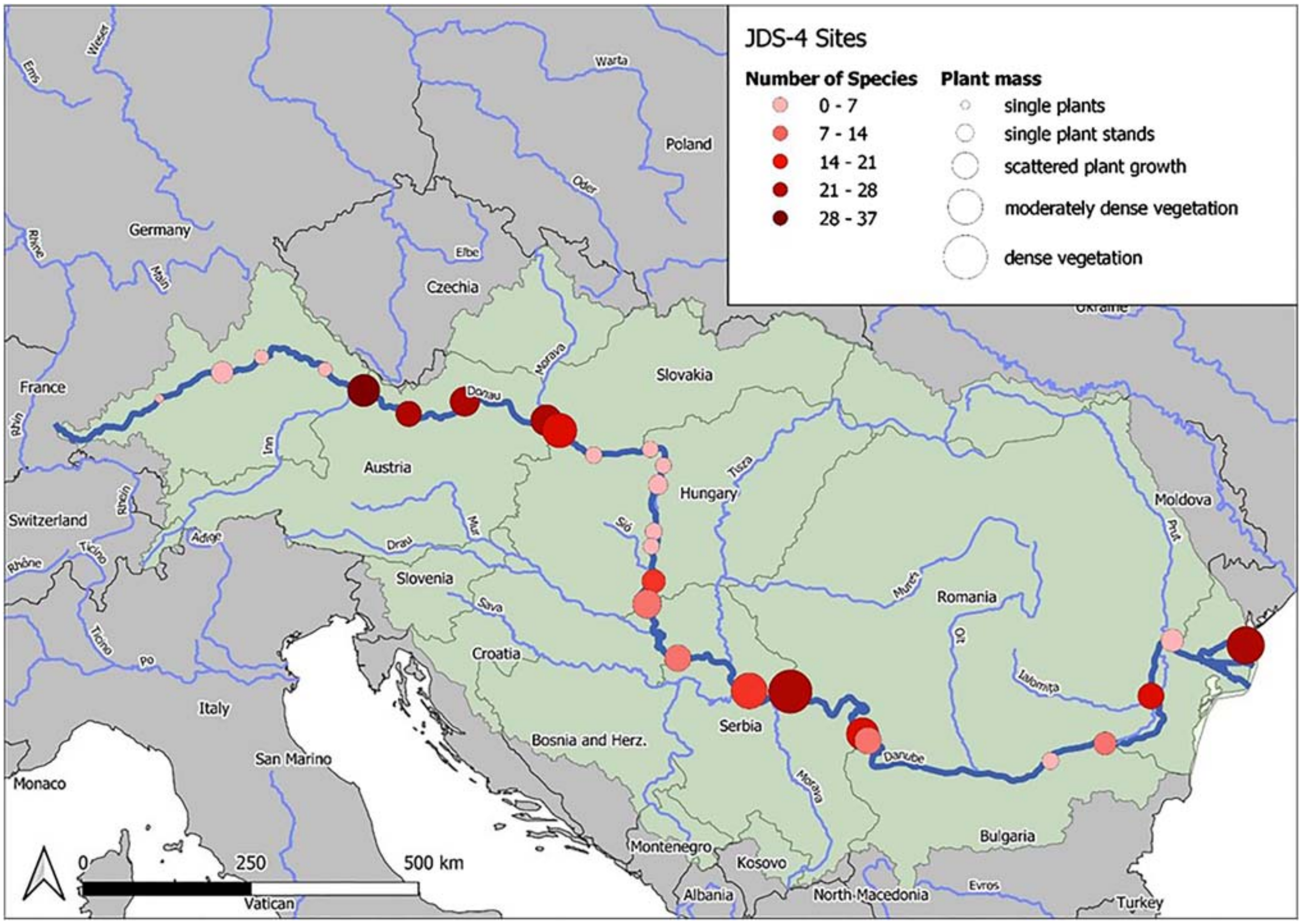

FIGURE 5.8 Macrophyta diversity along the Danube River—JDS4 survey sites.

from the Western Pontic to the Eastern Wallachian Danube (rkm 943-100). Floating-leaf and free-floating plants reach their peak growth in areas of the Danube with slow currents, such as the Pannonian Plain Danube, the Iron Gate Danube, and the Danube Delta. In these regions, these types of plants make up more than 50% of the overall plant mass.

In the Danube, influenced by the Alpine region (down to rkm 1807), the dominant species are the aquatic mosses *Cinclidotus riparius* and *Fontinalis antipyretica*, with other important moss species such as *Cratoneuron filicinum* and *Platyhypnidium riparioides*. Aquatic spermatophytes are not as prominent, with *Stuckenia pectinata* being the most important species. The halophyte vegetation along the banks is dominated by *Phalaris arundinacea* and *Lythrum salicaria*. In the Hungarian Danube Bend (rkm 1807—1497), *Cinclidotus riparius* remains the only noteworthy moss species. In addition to *Stuckenia pectinata*, other rooted spermatophytes such as *Myriophyllum spicatum* and *Elodea nuttallii* can be found in the water body. Floating-leaf species like *Potamogeton nodosus*, as well as numerous pleustophytes, including *Ceratophyllum demersum*, *Lemna minor*, *L. gibba*, and *Spirodela polyrhiza*, are also present. *Phalaris arundinacea* and *Phragmites australis* are frequently found on the river banks, along with important bank species like *Persicaria hydropiper* and *P. lapathifolia*.

As the Danube enters the Pannonian Plains (rkm 1497), aquatic mosses become scarce. Here, *Ceratophyllum demersum* is the most abundant species, along with mass occurrences of *Stuckenia pectinata*, *Potamogeton nodosus*, *P. perfoliatus,* and frequent occurrences of *Myriophyllum spicatum*, *Potamogeton crispus*, and *P. gramineus*. The pleustophyte species spectrum is widened with the addition of *Salvinia natans* and *Trapa natans*. The dominant species along the river banks are *Phragmites australis* and *Butomus umbellatus*. This situation remains stable in the further river's course. However, in the Delta region (rkm 100—0), some new species become important, including *Nuphar lutea*, *Nymphaea alba*, and *Stratiotes aloides* in the water, and *Typha angustifolia*, *T. latifolia*, and *Sparganium erectum* on the river banks.

Overall, *Ceratophyllum demersum* is the most abundant species in the Danube, observed at about 50% of all sites surveyed. *Myriophyllum spicatum*, *Stuckenia pectinata,* and *Spirodela polyrhiza* are additional important species, occurring at about 40% of all sites. Also, in terms of plant mass, *Ceratophyllum demersum* is the dominant species, followed by *Phragmites australis*, *Stuckenia pectinata*, *Potamogeton perfoliatus,* and *P. nodosus*.

The number of neophyte species increased from one JDS cruise to another. In 2001 (JDS1), only four species (*Elodea nuttallii*, *Solidago canadensis*, *S. gigantea,* and *Vallisneria spiralis*) were present. By 2007 (JDS2), three additional species (*Azolla filiculoides*, *Elodea canadensis*, and *Impatiens glandulifera*) were observed. In 2013 (JDS3), seven more alien species (*Bidens frondosa*, *Echinocystis lobata*, *Eclipta prostrata*, *Fallopia japonica*, *Impatiens parviflora*, *Lemna turionifera*, and *Symphyotrichum lanceolatum*) were found, bringing the total number of aquatic and semi-aquatic neophyte species along the Danube to 17 (including *Lemna minuta*, *Paspalum paspaloides*, and *Rudbeckia laciniata* from JDS4). Eight of these species are considered invasive in Europe, but only two (*Elodea nuttallii* and *Impatiens glandulifera*) are listed in the "List of invasive alien species of Union concern" (EU Regulation 1263/2017). The role and impacts of invasive alien species on native species and biodiversity are described in more detail in Chapter 9 of the book.

Macroinvertebrates

Aquatic macroinvertebrates, or macrozoobenthos, are the invertebrates that inhabit water ecosystems (bottom, water column, and surface, detritus, macrophytes, filamentous algae) at least in one period of their life cycle and which can be sampled using a net with a mesh size ≥200 μm (Rosenberg and Resh 1993). As one of the principal components concerning taxa richness, abundance, biomass, and functional relevance, they play an essential role in freshwater ecosystems, including large rivers such as the Danube.

The DRB is a "hot spot" for European macroinvertebrate biodiversity. Five international expeditions along the Danube and the main tributaries—AquaTerra Danube Survey in 2004 and Joint Danube Surveys (JDS1 to JDS4)—provided comparable data and point to the presence of about 500 macroinvertebrate taxa (Csányi and Paunović, 2006; Graf et al., 2008, 2015a; Literáthy et al., 2002; Očadlik et al., 2021). Sommerwerk et al. (2022) summarized the data from these surveys, which revealed the dominance of Diptera, Mollusca, Oligochaeta, Amphipoda, and Trichoptera in the macroinvertebrate communities along the Danube River in both quantitative and qualitative aspects. A longitudinal decrease in taxa richness was also identified, which may be explained by increasing anthropogenic pressures, and changes in natural conditions along the Danube River (Sommerwerk et al., 2022).

The comparative analysis of the riverine macroinvertebrate fauna based on the five surveys (i.e., period 2001−19) indicates that the Upper Danube (upstream of Kelheim, rkm 2415) is dominated by insect groups. Along the main channel, the highest diversity was recorded for Diptera, Trichoptera, Crustacea, and Mollusca, while the floodplain waters were dominated by Coleoptera, Trichoptera, Mollusca, and Odonata (Graf et al., 2008, 2015a).

Distribution patterns, environmental pressures, and aquatic macroinvertebrate species loss in the Danube and the main tributaries are generally discussed in Beermann et al. (2021); Graf et al. (2008, 2015a, 2015b); Lange et al. (2011); Očadlik et al. (2021).

More detailed investigations of aquatic macroinvertebrate fauna of the Danube, the main tributaries, and associated ecosystems are needed to complete our knowledge about the biodiversity of this important component of aquatic ecosystems. Investigations along larger geographical areas mainly focus on assessing the environmental status, thus providing information that is not entirely relevant for biodiversity assessment.

The macroinvertebrate community is shaped by numerous natural and anthropogenic factors, making the distribution analysis complex. Hydromorphological and chemical characteristics of water bodies, as well as biotic interactions and hydrogeological characteristics, the physical and the chemical characteristics of the water and the sediment, the substrate type, and interaction with other organisms—all of them characterized as key water management issues for the DRB (Sommerwerk et al., 2010).

Hydromorphological alteration and pollution by organic and hazardous substances (e.g., heavy metals, persistent organic pollutants) and pharmaceuticals, as well as the biological invasions, were found to be major factors influencing the decline in macroinvertebrate communities in the Danube River and associated wetlands (Sommerwerk et al., 2010; Paunović et al., 2015).

Fishes and lampreys

The Danube drainage is the most species-rich catchment in the western Palearctic when it comes to freshwater fishes and lampreys (Kottelat and Freyhof 2007). Altogether, we count 185 species in the Danube, with 156 native to the area, and 29 (18%) of them introduced (Freyhof, unpublished data). There are many more species than usually considered in the Danube (Schiemer et al., 2004), as 25 (16%) of the native species have not yet been described to science. These species are discovered by recent molecular assessment methods, usually by screening the fauna with mtDNA barcodes. The 25 undescribed species are primarily concentrated in genera such as *Phoxinus, Gobio,* and *Barbatula*; however, undescribed species also occur in other genera, and several additional species still need to be discovered. At the same time, some species recognized seem to be synonyms of others, asking for in-depth taxonomic revisions of several genera in the Danube (e.g., *Alburnoides*, *Alosa*, *Cottus*).

Out of the 156 native species, 58 (37%) are considered to be endemic to the Danube drainage (Kottelat and Freyhof 2007; Freyhof, unpublished data). Similar to undescribed species, most endemic species belong to genera inhabiting small headwater streams, such as *Phoxinus, Gobio,* and *Barbatula*, but also species from large and mid-sized rivers might be endemic, such as *Romanogobio, Rutilus,* and a few others. A hotspot of endemism are the subalpine lakes in Austria and Germany, where several species of *Coregonus* and *Salvelinus* are endemic, many of them extinct due to human alterations of lake ecosystems. Eleven (19%) of the 58 species endemic to the Danube have been assessed as being threatened by extinction (CR, EN, VU) following the IUCN criteria, which is the case in 22 (14%) of the species native to the Danube (Freyhof and Brooks, 2011). Four species, *Alburnus danubicus, Coregonus hoferi, C. renke,* and *C. bavaricus*, all endemic to the Danube, might be extinct. However, the diversity of *Coregonus* in the upper Danube was much higher, and there might have been about 30 species, principally undescribed and mostly extinct today (Freyhof et al., 2023). One species, *Scardinius racovitzai*, endemic to a hot spring in Romania, is now extinct in nature but survives in captivity.

This is also the case with sturgeons other than sterlet (*Acipenser ruthenus*) in the Danube, who all completely depend on stocking since their natural populations have been overfished already years ago. Enigmatic sturgeons, such as *Huso huso*, *Acipenser gueldenstaedtii*, *A. nudiventris,* and *A. stellatus,* still occurred in the Danube a few decades ago. While fishing on sturgeons in the Danube was regulated before 2007, all species were strictly protected after January 2007, when Bulgaria and Romania joined the European Union. However, all hopes for the protection of sturgeons vanished, as the European laws were not efficiently implemented, and fishing for sturgeons just continued on an illegal basis. Today, several "reintroduction programs" do their best to raise and stock sturgeons, who become mostly victims of illegal fishing within or outside the Danube. As this is the situation all over the distribution area of the species mentioned, strong efforts for the conservation of these species are needed, potentially with large ex-situ breeding stations, to stop the genetic erosion of species and their final extinction. Hydromorphological alterations and overexploitation of aquatic resources impacting fish biodiversity protection are further explained in Chapter 8 of this book.

While hosting a large diversity of native species, the DRB is also one of the hotspots of alien and invasive fish species in Europe. Altogether, 29 alien species are found in the catchment area, some belonging to the most invasive European species, such as *Pseudorasbora parva* and *Carassius auratus*, that are found virtually everywhere in the DRB. Others, such as *Perccottus glenii*, *Ameiurus nebulosus*, and *Lepomis gibbosus,* are widespread, but many others have small ranges, some of them as members of the families Cichlidae, Loricariidae, and Poeciliidae are restricted to few warm springs, into which aquarium hobbyists had released them. Three members of the family Xenocyprididae (*Hypophthalmichthys*, *Ctenopharyngodon*) are still released and escape from fish farms. Their establishment as self-reproducing populations in the Danube has been discussed many times but remains anecdotal until now. Overall, the Danube has a diverse fish fauna that is attractive to commercial and recreational fisheries. Therefore, the Danube remains one of the significant sources of alien species for Europe and North Africa. For example, companies establishing reservoirs for water storage and/or hydropower in North Africa often sell a complete package, including the fish to be stocked in the reservoir to improve fisheries. That practice has led to the establishment of several Danube fish species in those regions. Fish farms in the DRB also sell fish for stocking all over Europe, where these species are alien and massively impact the native faunas, as is the case, for example, in Italy. By this, the introduction of Danube fish species represents one of the major threats to the fish faunas of Southern Europe.

Altogether, the Danube hosts a diverse fish fauna that is still incompletely known, and much research is needed in the coming years. This includes the exploration of diversity (which species do we have and where are they distributed), the challenges of conservation (how to stop declines and extinctions, also under climate change scenarios), and alien species (assess the impacts and how to limit their expansions, inside and outside the Danube), as well as to set up a scientific monitoring system to detect change in fish diversity. The EU Water Framework Directive successfully established a regular monitoring for freshwater fishes in the EU. However, the data are widely dispersed at the subnational and national levels. There is an urgent need to link these data to analyze biodiversity change at the catchment scale. This will allow us to understand biodiversity change and help act accordingly within the diverse socio-economic and political landscape of the countries in the DRB.

Amphibians and reptiles

The distribution of amphibians and reptiles along the Danube River is relatively well studied and includes most species from the countries involved. Along the entire stretch of the Danube, 16 amphibian species and one species complex (water frogs of the genus *Pelophylax*), as well as 21 reptile species have been described. Of these, four species of amphibians and seven of reptiles occur only occasionally. A study of the distribution of herpetofauna along the Danube in Bulgaria identified 34 native species, of which 15 were amphibians, and 19 were reptiles, including one invasive terrapin (*Trachemys scripta*) (Popgeorgiev et al., 2019). Another long-term study of amphibians on an island in Vienna registered 11 out of the 19 amphibian species occurring in Austria (Kogoj, 1997). In Hungary, 14 out of 18 of amphibian species and 13 out of 16 species of reptiles (including the invasive *Trachemys scripta*) occur along the Danube (Puky et al., 2005). In Croatia, 11 out of 16 amphibian species have been recorded along the Danube (Mikuska et al., 2004), while 12 out of 21 species have been recorded for reptiles, including invasive species, *Trachemys scripta* and *Chelydra serpentina*, likely originating from pet shop collections (Mikuska et al., 2006; Mikuska, unpublished data). Among the threatened species of Serbia, out of 16 species, nine occur along the Danube, including the introduced gecko *Mediodactylus kotschyi* (Tomović et al., 2015), while for amphibians, seven out of 10 species included in the Red List are present along the Danube in Serbia (Kalezić et al., 2015). Another study along the Romanian side of the Iron Gates in 2012 inventoried 16 amphibian species (out of 20 occurring in Romania) and 17 reptile species (out of 23 occurring in Romania). Four species reported before 1971, when the Iron Gates Dam was built, were no longer recorded (*Pelobates balcanicus, Triturus cristatus, Lissotriton vulgaris,* and *Zootoca vivipara*), and a range reduction was observed in 10 amphibians and 13 reptile species, while the rest expanded their ranges, taking advantage of the new habitats created after the reservoir had been formed (Stănescu et al., 2015).

One species of newt, the Danube Crested Newt (*Triturus dobrogicus*), is endemic and occurs only along the Danube River and its tributaries (Arntzen et al., 1997). Due to differences in morphology, two subspecies were considered, one in the Pannonian Plain and one downstream of the Iron Gates (Litvinchuk and Borkin, 2000), but this was not confirmed by later studies that consider it a monotypic species (Wielstra et al., 2016). Also, the taxonomic status of tree frogs of the *Hyla arborea* complex along the Lower Danube is still uncertain, with *H. orientalis* probably occurring downstream of the Iron Gates, while *H. arborea* is found upstream of the Iron Gates (Dufresnes et al., 2016).

Birds

The central position in the Black Sea/Mediterranean Flyway and size of the Danube River in Europe favors major stopover, breeding and wintering sites for millions of birds that are attracted along the river and its floodplains (Sommerwerk et al., 2022) and connecting birds from Northern Europe and West Siberia to sub-Saharan Africa. Close to 400 bird species have been recorded along the DRB so far. The first comprehensive study of the bird fauna along the Danube has been published by Ciochia (2001), who listed 388 bird species, including 241 breeding. A recent study confirmed the breeding of 258 bird species along the Danube River (Keller et al., 2020) with significant breeding populations of water and wetland-related species (Sommerwerk et al., 2022).

Only a handful of bird surveys cover the entire river length. One of the longest-running (since 1967) and most comprehensive (since the 1990s) surveys is the International Waterbird Census (IWC) under the organization of Wetlands International (https://www.wetlands.org/knowledge-base/international-waterbird-census/). Carried out in mid-January under standardized protocols, it covers all water-related species that are wintering on the river or its floodplains (including man-made habitats such as reservoirs or fishponds). Data collected are used to establish size estimates and trends of waterbird populations (Nagy and Langendoen, 2020), and they have been regularly published on the Waterbird Populations Portal since 2012 (https://www.wetlands.org/knowledge-base/waterbird-populations-portal/), as well as nationally (e.g., https://bspb.org/en/results-of-the-46th-midwinter-waterfowl-census-in-bulgaria/).

A danube-wide census of breeding populations of river-related birds, such as Little-ringed Plover *Charadrius dubius* and Sand Martin *Riparia riparia,* that serve as indicators for dynamic hydromorphological processes, was carried out in 2011 (DanubeParks, 2012) and 2021/2022 along Mura, Drava, and part of Middle Danube (Podgorelec et al., 2022). The white-tailed eagle *Haliaeetus albicilla* is another iconic and flagship species whose breeding and wintering populations along the Danube were censused (Probst and Gaborik, 2012; Probst et al., 2014).

The diversity of bird species and their abundance along the Danube River are the main criteria for designating protected areas under EU Birds Directive (2009/147/EC; https://eur-lex.europa.eu/eli/dir/2009/147/oj). Until now, 79 Natura 2000 Special Protected Areas (SPAs) and Important Bird Areas (IBAs) were designated, covering over 1,514,435 ha in total (Sommerwerk et al., 2022). While the remaining free-flowing parts and active floodplains of the Danube River are included in the network of protected areas, two large sites stand out. Firstly, in the Middle Danube, in the triangle of Hungary, Croatia, and Serbia, three protected areas (Béda-Karapancsa of Danube-Drava National Park (HU), Kopacki rit Nature Park (HR), and Gornje Podunavlje Special reserve (SR), covering in total 117,316 ha represent the best-preserved part of the meandering river and hearth of the transboundary Mura-Drava-Danube Biosphere reserve (see Chapter 12). It supports the densest breeding population of the White-tailed Eagles (*Haliaeetus albicilla*) in the whole of Europe, with over 120 breeding pairs. And secondly, in the Lower Danube, the Danube Delta, covering over one million ha, stands out as the largest and most important site, with 365 bird species recorded so far (Marinov et al., 2023). It supports the breeding of up to 48% of the total Palearctic breeding population of the Great White Pelican (*Pelecanus onocrotalus*) and thousands of pairs of different heron, ibis, and cormorant species (Marinov et al., 2023). The Danube Delta, linking the Danube River and the Black Sea is separately described in Chapter 6 of this book.

Mammals

Around the Danube, mammals have to deal with wetlands like riparian meadowlands and floodplain forests, which are frequently flooded areas. Typical for floodplain forests is that they can regenerate naturally, they are old-growth forests and are highly heterogeneous (Suchomel et al., 2020). This is a good basis for biodiversity, but the isolation and fragmentation of the natural habitats are some of the major perils to biodiversity in the Lower Danube (Popov et al., 2019). Thus, the increase in fragmentation reduces the ecological connectivity of high-value ecosystems along the river (Frank, 2017). This mainly affects large mammals like the wolf (*Canis lupus*) or the red deer (*Cervus elaphus*) that need large natural habitats because adult individuals migrate.

The natural occurrence of small mammal species, like the very rare Mehelyi's root vole (*Microtus oeconomus mehelyi*), is difficult to assign along the river. According to currently available data—Mehelyi's root vole is restricted to the middle sections of the Danube (Gubányi et al., 2009). The shrunken wetland habitats on both sides of the Danube impede the migration of large and small mammals.

An invasive mammal species that occurs along the Danube is the coypu (*Myocastor coypus*). It is a semiaquatic rodent native to South America. Mainly due to fur farming, it is distributed in Europe. Multiple sightings were made in the Danube floodplains in Austria, Donau-Auen National Park (Schertler and Essl, 2022), Serbia and Romania, including the Danube Delta (e.g., https://observation.org/species/1490/).

The Danube reaches its climax regarding mammals at the end of its path, the Danube Delta. The Danube Delta is one of the most extensive wetlands in Europe, and it supports a diverse mammal fauna with 54 species (Kahl 2018), including species of high conservation value, such as the European mink (*Mustela lutreola*) or the European beaver (*Castor fiber*). Intensively hunted for fur and castoreum oil, the European beaver was extinct for over a century in the Danube region; after reintroductions, it returned to most of its former range (Bajomi, 2011; Halley et al., 2012).

Interpretation of species lists: how accurate and reliable are they?

The systematic collection and analysis of biodiversity data and ecosystem services is essential. It contributes to the understanding of the causes of biodiversity loss and is needed to analyze trends and evaluate intervention measures and policies (Sommerwerk et al., 2021). Species lists are generated to assess, how many species occur in a particular area. Primarily, they are established to provide easily understandable overviews and qualitative estimates of taxa. However, there is always the chance that species lists are incomplete, as presented by Gómez de Silva and Medellín (2001). They found that in scientific literature, species lists vary in completeness. A limitation of species lists in scientific literature lies in the fact that they were made by field observers with different goals and levels of expertise, using different methods over variable lengths of time (Gaston, 1996). Thus, species lists are snap-shots but are, however, often used for years because monitoring is time-consuming and cost-intensive. These outdated lists then often provide the basis for species conservation to estimate the risk of extinction and provide arguable results. Apart from proper sampling, the completeness of species lists is further troubled with correct identification across all possible taxa. Additionally, most monitoring protocols are designed to maximize time and cost-effectiveness; thus, such general studies do not cover hard-to-find, cryptic, or rare species.

Finally, there is also an issue of species that are taxonomically hard to identify or those that have not yet been properly described to science. With quite a share of European biodiversity, particularly invertebrates and fungi, still unknown to science, there is an everlasting need for new taxonomists. However, the trends are exactly the opposite, and the number of skilled taxonomists is decreasing across the continent (Buyck, 1999; Drew, 2011; Engel et al., 2021; Hochkirch et al., 2022). The leading causes identified for this problem are (1) the fact that taxonomy is rarely recognized as a science, in particular by decision-makers, (2) the reduced number of highly specialized scientists, with solid theoretical and practical backgrounds, (3) the numerous barriers encountered for collecting and storing specimens, such collections representing the scientific basis of this science (Engel et al., 2021). This is coupled with a global lack of academic education in taxonomy and of properly trained taxonomists, shortage of professional positions and research grants, associated devaluation of taxonomic publications (taxonomic treatments, monographs, and species publications are rarely cited outside taxonomy), and inability to accomplish advancements in professional carrier compared to other fields of studies. It is widely acknowledged that novel methodologies (e.g., DNA barcoding or photo identification) are inappropriate as the sole basis for taxonomic work, which consists of delimiting species and ascertaining their relationships (Engel et al., 2021). Although this creates the impression that taxonomic work is not necessary and that career prospects in systematics and taxonomy would be lost, graduates with solid knowledge of species currently have very good opportunities on the job market, for example in planning offices and authorities. In addition, specialist societies, associations, natural history museums, zoological, and botanical gardens currently offer expertise and career prospects in this area. Nevertheless, taxonomic expertise is rapidly disappearing from the educational landscape (Sommerwerk et al., 2021).

Biodiversity data were traditionally used for conservation purposes to proclaim protected areas, both nationally and internationally. For example, over 79 Natura 2000 areas or important bird areas were designated along the Danube (https://ec.europa.eu/environment/nature/natura2000/awards/natura-2000-network/index_en.htm). Moreover, 25 Ramsar sites (https://www.ramsar.org) and four UNESCO Biosphere reserves (https://en.unesco.org/biosphere/eu-na) are established. These protected areas are usually designated based on species lists and the presence of viable populations of keystone, flagship, or umbrella species (primarily birds and mammals) or other strictly protected or threatened species (reptiles, amphibians, fish, insects, molluscs, or crustaceans). Those criteria have been the basis for the foundation of the EU Birds and Habitat Directives and the designation of the pan-European ecological network. Another essential criterion, developed primarily for birds and mainly used for the designation of Ramsar sites and Special Protected Areas under the Birds Directive, was based on abundance criteria (e.g., that particular site supports 1% of breeding, passage, or wintering flyway population and/or large concentrations, e.g., over 20,000 individuals of the given species) (https://www.ramsar.org/sites/default/files/documents/library/ramsarsites_criteria_eng.pdf, Balmer 2002). Protected areas along the Danube River, and their management and controversies over their sufficient extent, positions, and proper management are further described in Chapters 12 and 13.

Another important use of biodiversity data arises from the EU Water Framework Directive (EU WFD, 2000) where data are used for the assessment of the good ecological status of freshwater water bodies, as well as to serve as indicators for economic activities and to monitor the implementation of public policies. Instead of simple species lists, good ecological status is assessed based on functional communities of algae, phyto- and zooplankton, macrophytes, macroinvertebrates, and fish. In the last decades, there has also been an attempt to use some bird species (such as Little Ringed Plover or Sand Martin) as indicators for dynamic hydromorphological processes along the river (DanubeParks, 2012; Podgorelec et al., 2022). Transnational management of water bodies in the Danube and Black Sea region and the status of protected areas along the Danube River are additionally described in Chapter 11.

Methods to collect and identify species

For monitoring purposes, species need to be identified correctly. For most of the vertebrate species, identification keys and standardized methods for their studies were developed a long time ago (e.g., Bibby et al., 2000). For mammal species in Europe, identification guidelines were developed (Handbook of the Mammals of Europe, series editors Hackländer and Zachos, 2020; Niethammer and Krapp, 1978-2005). For long-term monitoring of small mammals, sorting out barn owl pellets provides effective tools to follow the dynamics and evolution of rodents and shrew populations over large areas (Stefke and Landler, 2020). Pellets are sorted out, and the entire skull and mandibles or their remains are used for identification. Several identification keys exist (März and Banz, 1987; Jenrich et al., 2012; etc.). For bats, detectors enable a noninvasive monitoring of species, facilitating observations that would be difficult to obtain by classic capture-release methods or roost inspection. However, the method has also occasional drawbacks for the species that cannot be identified from their calls; in such cases, acoustic classification can be ambiguous or impossible (Russo and Voigt, 2016).

Standardized methods and sampling protocols have also been developed for collections of invertebrate species, for example, using pitfall traps as a standard method to collect invertebrates from the banks or the floodplain (Anderson et al., 2013). Further, we describe several novel methods that have been enabled by technological advances in the past few decades.

Acoustic biodiversity monitoring

Passive acoustic monitoring (PAM) provides unprecedented opportunities for faunal surveys due to the automated acoustic sensors allowing high-volume and long-term data collection, which makes them increasingly popular in biodiversity inventories (Sugai et al., 2020). The high costs and constraints restricted their use to several taxonomic groups (e.g., bats, birds, cetaceans) and often to small-scale studies, until recently, when low-cost, open-source sensors became affordable, expanding rapidly the access to PAM technologies (Gibb et al., 2018).

With the exponential increase of soundscape recordings, various new computational methods for acoustic monitoring using global soundscape indices and automated species identification are being developed. Acoustic indices provide simple statistical summaries of the spectral and/or temporal distribution of energy in an acoustic recording. Machine learning can provide a semi-automated classification method for terrestrial soundscapes and integrate compound indices with time series classification (Scarpelli et al., 2021). In the meantime, species detection by fully automated methods remains problematic, considering that a comprehensive, manually labeled call library is required for training data, but creating such a library is often confined by time, resources, or data availability. These limitations can be circumvented using active learning methods to inventory species in a novel habitat (Eichinski et al., 2022).

Environmental DNA (eDNA)

A successful method for species diagnosis is DNA analysis. A precondition for DNA fingerprints is that the species is already sequenced, and the sequencing is based on the species that have been determined. Genetic databases were designed to provide genetic data and comprehensive DNA sequence information (https://www.ncbi.nlm.nih.gov/genbank/). In wild nature, noninvasive materials like hairs or feces are collected for eDNA analysis. Especially with elusive animals, like the European wildcat (*Felis silvestris*) or the European otter (*Lutra lutra*), it is a favorable method (Prigioni et al., 2006; Steyer et al., 2013).

The possibility of detecting species using eDNA in water, soil, and even air samples has a huge potential for gaining insight into the ecology and conservation of species (Goldberg et al., 2015; Roger et al., 2022; Bohmann and Lynggaard, 2023). Thus, eDNA methods could significantly increase the data available on the occurrence of rare or endangered species and allow for early detection of invasive species. However, considering the sensitivity of eDNA methods, increased awareness and attention to quality assurance and quality control protocols is needed (Goldberg et al., 2016). The use of eDNA for the detection of aquatic species was tested for the first time along the Danube River during JDS4 on fish, macrozoobenthic, phytobenthic, and sediment communities (Weigand and Astrin 2021; Pont et al., 2021; Zimmermann et al., 2021; Cordier et al., 2021). The evaluation of the results was summarized by Weigand (2021).

Camera traps for vertebrates

Automated camera traps (CTs), with increasing possibilities of immediate data transfer via GSM network, are becoming popular means of studying primarily terrestrial vertebrates (Trolliet et al., 2014; Scheiblechner and Teubner, 2023). They are used for both large and small mammals (Gracanin and Mikac, 2022), but they can also provide data on other terrestrial vertebrate species. They are particularly beneficial for studying rare or elusive animals like Otter or Eurasian Beaver (*Castor fiber*) that are difficult to observe in natural habitats due to their solitary nature or nocturnal behavior. Camera traps are nowadays used to monitor the presence/absence of certain species in the area, estimate population density for conservation purposes, and study habitat use and behaviors (Trolliet et al., 2014; Scheiblechner and Teubner 2023). If a studied species possesses individual traits (fur marks, injuries, coloration pattern, etc.) or if they are artificially tagged (particularly birds such as eagles, storks, spoonbills, etc.), this technology would allow the identification of the individual animal. A plethora of software is available for photo identification (e.g., Wild.ID (https://www.wildid.app/), I^3S (https://reijns.com/i3s/). Other methodological issues, detection biases, and other risks for the use of camera traps are described by Caravaggi et al. (2020).

Aerial and aquatic surveys using drones for large vertebrates

Aerial survey is considered the most effective technique for monitoring extensive, inaccessible areas, such as, for example, large river floodplains and offshore waters. Aerial surveys using fixed-winged aircraft for monitoring large vertebrates, including waterfowls, have been used since the late 1950s (Komdeur et al., 1992). The aerial survey proved to be a valuable tool in determining waterbird communities, their species richness and abundances (Kingsford et al., 2008; Kingsford and Porter 2009). However, this method has also shortcomings, mainly due to the high expenses involved and high speeds of surveys, leading often to relatively low accuracy of identification and counting. In particular, in wooded wetlands, aerial surveys tend to underestimate abundance and species richness significantly due to treetop cover (Kingsford et al., 2008).

With the development of new technologies, unmanned aerial vehicles (UAVs) or dynamic remotely operated navigation equipment (DRONEs) rapidly become part of environmental monitoring and management applications (Schad and Fischer, 2022). Nowadays, drones are affordable, highly mobile aerial platforms that enable researchers to study individual behavior, species abundance and distribution, track population dynamics, build digital terrain models of ecosystems, as well as assist conservation efforts (Ivošević et al., 2015; Mikuska et al., 2015; Marchowski, 2021). Drones were primarily used to study large vertebrates, particularly mammals and birds, but recent advances in technology allow their use for insect sampling (Ivošević et al., 2017; Madden et al., 2022), study reptiles (Fagundes et al., 2020), or delivery of anesthetics or medicines to wild animals (Brinkman and Garcelon, 2020). Apart from developing the technology to expand the field of use, security reasons and disturbance impacts on wildlife were always the primary focus of usage of this tool (Vas et al., 2015; Lyons et al., 2018).

Animal telemetry and GPS tracking

The development of Global Positioning System (GPS) technology for tracking animals began in 1991 and with its spread for commercial purposes, a whole new chapter has been opened in animal studies (Rodgers, 2001). The use of GPS telemetry in wildlife research has increased worldwide, this method enabling the acquisition of spatial and temporal data of animal location with enhanced resolution. At first, due to the weight of battery size, costs of GPS units, and data provisions, they were used for studies on mammals and large birds to determine their distribution and movement. However, rapid development in micro-GPS receivers and integration of solar panels as the primary power supply source, battery endurance, and GSM mobile technology quickly led to a decrease in their size and weight (geolocators weight could be down to 0.2 g), enabling their use on small animals, from songbirds to rodents (McMahon et al., 2017). Among many examples, such devices integrate several different sensors (depth, speed, pressure, etc.), enabling animal behavior and habitat use studies. GPS data can provide results on migration patterns, size of a territory or home range, and activity of single individuals or a group (Kojola et al., 2018; Henrich et al., 2021). Nowadays, telemetry technology also enables studies of purely aquatic (fish) or predominantly aquatic animals such as beavers or otters (Holtgren and Auer, 2004; Quaglietta et al., 2012; Graf et al., 2016; Honţ et al., 2018).

Citizen science in biodiversity monitoring

There is a long tradition in volunteer research. Data collection by volunteers has played a key role in government reporting for decades and are relevant for the development of research questions and in applied research (Sommerwerk et al., 2021). Zoological and botanical societies as well as environmental associations also rely on voluntary commitment. Nowadays, the involvement of citizens is becoming increasingly significant. In the context of scientific activities, volunteer work is called “citizen research” or “citizen science.” This approach focuses on joint research with actors from civil society (Sommerwerk et al., 2021). Successful integration of citizen research into biodiversity monitoring cannot only ensure the transparency of research approaches and increase mutual recognition between science and volunteer work but also strengthen social appreciation of biodiversity and the understanding of scientific approaches and methods. In this way, based on a broad spectrum of ecological expertise and different perspectives on nature, research results can be better understood and classified, and applied solutions to environmental problems can be found (Sommerwerk et al., 2021). Furthermore, growth of leisure activities in nature has led to the development of nature observations by ordinary people. While it primarily started with bird watching (as birds are one of the most visible parts of biodiversity), it quickly expanded to other eye-catching taxa like flowering plants, butterflies, or dragonflies. World-wide interest in nature and increasing mobility led to the development of online platforms for observation reporting and data storage (e.g., Observation.org, iNaturalist.org, e-bird.org, etc.) and citizen science projects (Pocock et al., 2014). Citizen science presents an alternative to traditional systematic protocols in ecological monitoring with the advantage of very cost-effective (mass) data collection using recent technological advances (smartphones, cameras) that make data collection straightforward (Pocock et al., 2014). Citizen science projects and data collection have been applied to various conservation purposes, from mapping species diversity and estimating species dynamics to ecological monitoring or studying climate change ecology (Arazy and Malkinson, 2021). Disadvantages of data collection by citizens are primarily related to biases concerning correct species identification or unstructured data collection affecting the temporal and spatial scale (Arazy and Malkinson, 2021; Callaghan et al., 2021a, 2021b). While citizen science can collect large amounts of data on easy-to-identify species, it has limited practical meaning when dealing with taxa that require complex taxonomic knowledge and methods for proper species identification (e.g., most invertebrate groups, algae, etc.).

Pressures on biodiversity and challenges posed by water scarcity due to climate change

Due to its central position and the size of the river basin that covers 10% of continental Europe, the Danube River has facilitated human population development and shared its consequences throughout history. Nowadays, an estimated 79 million people live in this basin and depend on surface water and groundwater for drinking water supply, energy production, agriculture, and transport (ICPDR, 2021).

Major changes that negatively affected the Danube River and its floodplains, habitats, and biodiversity started with the Industrial Revolution and are continuing until the present. Morphological alterations of the river for flood protection and navigation purposes started in the 18th century and reached their peaks in the 19th and 20th centuries. Deterioration of natural river morphological conditions was performed by river straightening and re-profiling, bank reinforcements, riverbed stabilization, and flood defense system construction. Consequently, in the Upper Danube, the total river width decreased on average by 39%, in the Middle Danube by 12%, and in the Lower Danube by 4%, respectively (ICPDR, 2021). Additionally, the length of the river was reduced by about 100 km (−11%) in the Upper Danube, about 30 km (−4%) in the Middle Danube, and 1% in the Lower Danube (ICPDR, 2021). Even more dramatic changes were made by disconnecting the river from the surrounding floodplain—compared to the 19th century, less than 19% of the former floodplain area (7845 km^2 out of once 41,605 km^2) remain connected to the Danube River (ICPDR, 2009). Nowadays, only 144,659 ha of wetlands/floodplains have been identified to have reconnection potential in the whole river basin (ICPDR, 2021; see also Chapters 15 and 16), with the note that not all perspective wetlands/floodplains were reported by national authorities (e.g., in Croatia—for further information see: Schwarz, 2016; Glatz-Jorde et al., 2021). Further hydrological alterations of the Danube occurred from constructing impoundments that alter the river's upstream and downstream flow conditions. Currently, 26 such large barriers, constructed primarily for electricity production, represent critical hydrological pressure causing significant alteration on approximately 1069 km (37.4%) of the whole river length (ICPDR, 2021). As a consequence, barriers interrupt longitudinal connectivity along the river including the downstream transport of sediment and the prevention of upstream migration of aquatic organisms, particularly fish. The impact of key drivers that result in significant pressures along the DRB is summarized in Fig. 5.9. Flood protection measures are the main key driver that affects 98%−100% of river length, followed by hydropower development (85%−94% of river length), dredging (31%−59%), navigation (22%−55%), and agriculture (21%−48%) (NARW, 2018).

The above-mentioned alterations and uses had profound negative impacts on the overall biodiversity, primarily through habitat loss, degradation of habitat quality and its diversity, increased pollution, and unsustainable use of the population of

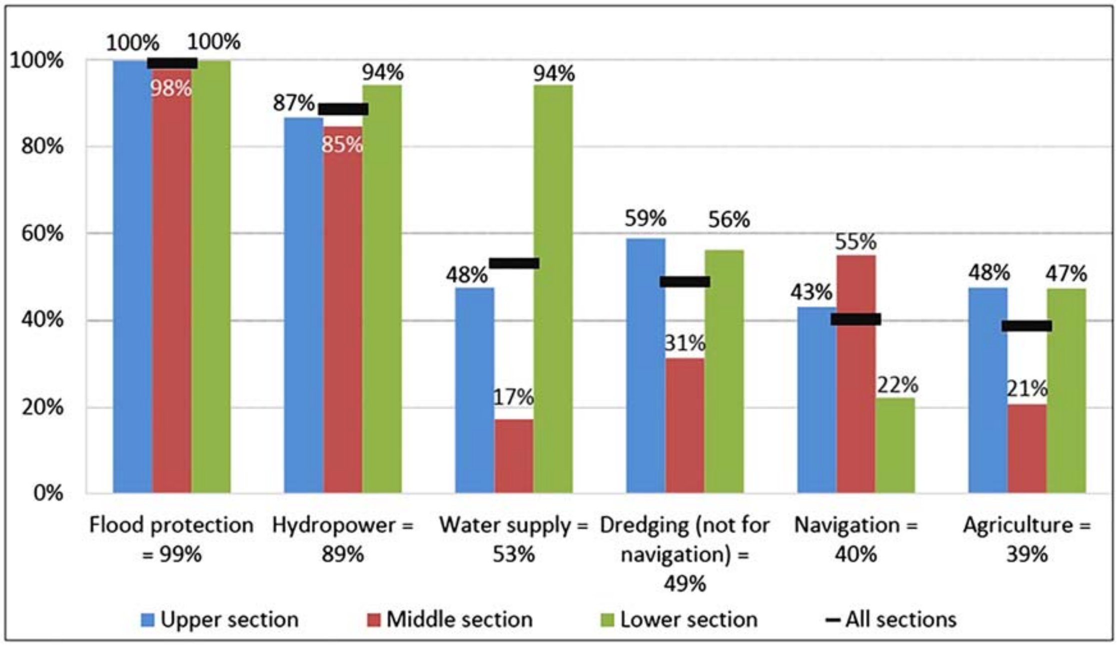

FIGURE 5.9 Percent of river stretch to absolute length affected by key drivers on the Upper, Middle, and Lower Danube River section and on all sections. *Courtesy of NARW (2018).*

aquatic animals, primarily fish. Consequently, in many stretches biodiversity severely decreased compared to the original state, and the number of species was driven to partial extinctions. For example, loss of river dynamics and natural morphological processes is very well represented by two bird species: Little Ringed Plover (*Charadrius dubius*) that breeds on gravel and sand islands/banks, and Sand Martin (*Riparia riparia*) that nests in steep natural riverbanks that are the result of active natural erosion of rivers. Due to considerable human pressure, the formerly widespread distribution of both species is now limited to the remaining sections with sufficient river dynamics (DanubeParks 2012; Liška et al., 2015). Numerous other plant and animal species faced population decline and partial extinctions due to habitat loss related to the disconnection of floodplains from the river and their conversions to agriculture and construction sites. Interruption of longitudinal connectivity negatively affected the natural migrations of aquatic species, particularly fish. An example is the construction of Iron Gate I and II, which prevented the migration of sturgeons to their main spawning sites in the Middle Danube and caused their extinction upstream of the dams (Bloesch et al., 2005). Similarly, the construction of barriers in the Upper Danube led to the extinction of Danube Salmon (*Hucho hucho*) in affected rivers and tributaries and a large part of their former distribution (Witkowski et al., 2013; Weiss et al., 2018). In all other areas, populations of native fish species are seriously depleted, and fish communities are disturbed (Bammer et al., 2015).

Detailed descriptions of hydromorphological alterations and overexploitation of aquatic resources are provided in Chapter 8, while the impact of invasive alien species is presented in Chapter 9.

Other far-reaching consequences concerning water-dependent ecosystems and biodiversity are related to human-induced climate change and predicted lack of water. Different climate simulation models nowadays generally agree on a significant increase in temperature by the end of the 21st century, with the most pronounced increase in the south-eastern part of the DRB (EEA 2015; Pistocchi et al., 2015; Probst and Mauser, 2023). This would be coupled with a general increase in precipitation, mostly pronounced in the upstream parts of the catchment (Pistocchi et al., 2015). Discharge seasonality will shift toward increasing winter and decreasing summer runoff with low flow increase along the Lower Danube (Probst and Mauser, 2023). With the changes in seasonal runoff, the effects of climate change are likely to lead to a reduction in water availability, reduced snow storage, and increased evapotranspiration (Mauser et al., 2018). Droughts and low flow will likely become more intense, longer, and frequent, particularly in the Middle and Lower Danube (Mauser et al., 2018; Sušnik and Moderc, 2019). Water scarcity would have severe consequences, decreasing water quality during low flows, reducing the floods needed for the reproduction of water-dependent plants and animals, and causing the long-term general shift in biodiversity and ecosystems from aquatic to more terrestrial flora and fauna. Furthermore, water-dependent sectors such as agriculture, forestry, navigation, and energy production from hydropower plants would also suffer under projected conditions and water demand (Mauser et al., 2018; Sušnik and Moderc, 2019) and make overall water management in the whole river basin more challenging. For more details, see Chapter 10.

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Ecohydrology from Catchment to Coast

The Danube River and The Western Black Sea Coast

Complex Transboundary Management

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Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands
125 London Wall, London EC2Y 5AS, United Kingdom
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States

ISBN: 978-0-443-18686-8

For information on all Elsevier publications visit our website at https://www.elsevier.com/books-and-journals

Publisher: Candice Janco
Acquisitions Editor: Maria Elekidou
Editorial Project Manager: Aleksandra Packowska
Production Project Manager: Paul Prasad Chandramohan
Cover Designer: Christian Bilbow

Typeset by TNQ Technologies

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Series Editors Eric Wolanski, Mike Elliott, and Robert H. Richmond

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