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Danube migratory fish habitat manual

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FOREWORD

The Migratory Fish Habitat Manual is developed as part of the project **MEASURES** - **M**anaging and restoring aquatic **E**cologic**A**l corridors for migratory fi**S**h species in the dan**U**be **R**iv**E**r ba**S**in as part of the Interreg Danube Transnational programme. The Manual is compiled from the experience of the project partners in application of different research methods of the migratory fish habitat.
Descriptions of the migratory fish (habitats) research methodologies are dominantly based on the examples tested during the MEASURES project.

Chapter 2 provide a description of the migratory fish species in the Danube Region. Descriptions provide information about distribution, species description, species identification, species ecology, reproductive biology, threats and conservation status as well as emphasis on missing/unknown data.

Detection of potential key habitats and their spatial extension is explained in Chapter 3 with numerous examples collected and tested be the project partners.

The Verification of habitats by presence of species is tested from standard methods as capturing, tagging, electrofishing, telemetry and e-DNA. The methodology is focused on adult and juvenile specimens. Moreover, the researches of habitats related to different life stages as well as seasons are described in Chapter 4.

Final chapter, provide information on the advantages and limitations of different methods used in migratory fish habitat assessment and estimation of the costs of the methods for habitat assessment methods.

Publication of this manual was possible thanks to the financial support offered by the project "Managing and restoring aquatic Ecological corridors for migratory fish species in the Danube River Basin" coordinated by the University of Natural Resources and Life Sciences, Vienna (BOKU). This project is implemented through the Danube Transnational Program and co-funded by European Union funds (ERDF, IPA, ENI).

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Content

CHAPTER 1.

Historical introduction

Radu Suciu

The Danube River was well-known for its rich fisheries of which long-migratory sturgeons stood out by size and the quality of their flesh and eggs (Marsigli, 1744; Heckel & Kner, 1858; Herman, 1887; Antipa, 1905, 1909, and 1916; Hensel & Holčík, 1997; Friedrich, 2018). Sturgeons were not the only migratory fish species in the Danube, but their tragic history is closely linked and was possible due to the slow progress of knowledge on their life cycle and habitat requirements. Characteristic of this was that up to the mid-20th century most of the information on migratory fishes in the Danube was based on data from fishermen. The most striking question when studying migratory sturgeons of the Danube River is how was it possible that for centuries, fishermen and ichthyologists alike have not been able to link the massive arrival of Beluga sturgeons, the "ancestral fish species of the Hungarian fishery" (Herman, 1887), in the Hungarian Danube to their persistence and reproduction.

No wonder that about 250 year earlier, Luigi Marsigli (1744) wrote that local fishermen believe that Stellate sturgeons were coming from the Black Sea to the Danube cataracts (Iron Gates) and were swimming from here to the Sea of Marmara through a subterranean canal (Marsigli, 1744). However, some of the information in Marsigli's book on Danube Fishes (1744), based on observation by fishermen, such as the strict habit of sturgeons to pass upstream and downstream by all of them always taking the same route in the river, was and still is valid today. About 100 hundred years later, the information mentioned by prominent ichthyologists of the time were truly astonishing, like for example the one on Great sturgeons from the book "Freshwater Fishes of the Austrian Monarchy": "Nach Angabeverlässlicherungarischer Fischer steigter von Anfang Märzbis Ende Mai, dannwieder von August bis Dezember die Donaustromaufwärts, *ohneaber je darinzulaichen* (According to reliable Hungarian fishermen, it rises upstream in the Danube from the beginning of March to the end of May, then again from August to December, *but never spawns in it*)" (Heckel&Kner, 1858, p. 369).

Not surprisingly the knowledge and terms used by Hungarian fishermen for the various types of fish related parts of the river was indeed astonishing. According to the "Book of Hungarian Fishery" by Herman Oto (1887, p. 408), they were using 18 different words to describe river banks: "A part: lankás, eresztős, meredek, omlásos, dülö, padmalyos, odvas, szirtos, csorba, bokros, gyökeres, csöntörgés, sima, fövenyes,köves, sziklás, agyagos, süppedékes (The bank: sloping, descending, steep, crumbling, seating, paddy, cliffy, rocky, chipped, bushy, rooted, ridgy, smooth, sandy, stony, rocky, clayey, plain). Also, Hungarian fishermen were using 12 specific words to distinguish the different types of river bottom substrate: "A fenék : tiszta, gödrös, fövenyes, iszapos, kavicsos, köves, atkás, gamóczás, gyökeres, uszadékos, zátonyos, gübbens (The bottom: clean, pitted, sandy, muddy, pebble, stony, with entangling obstacles, rooted, silty, reefy, tuberous). Despite this detailed knowledge related to fishing sites, 19th century Hungarian fishermen were ignorant about the primary reasons why sturgeons were migrating every year from the Black Sea over 1700 km to the Danube, as far as Komárom / Komárno or even Pozsonyi / Bratislava. As contemporary Hungarian ichthyologists (Guti & Gaebele, 2009) wrote: "One of the obstacles to effective active conservation measures is that at present, the development of indigenous stocks of sturgeon can only be characterized by indirect fishing data series. We do not have reliable information on habitat use, migratory pathways, distribution of key habitats, morphological and hydraulic characteristics of populations, size of populations, and so forth".

"The habitat of a particular species is the *set of environmental conditions* that satisfy the requirements of the individual organisms that make up that species. It is an abstraction that is centered on the elements of the environment that the organism requires *for its persistence and reproduction.* A species may occupy multiple biotopes if all of them satisfy its environmental requirements". This is the way the emerging science of autecology defines habitats (Walter & Hengeveld, 2014). Noteworthy is that this definition from 2014 clearly links habitats to the persistence and reproduction of individuals of a species. Also, autecology clearly distinguishes between epiphenomenon, as a side-effect, which can be interpreted only by understanding the primary cause of animal behavior. Studying the epiphenomenon in itself can be extremely misleading (Walter & Hengeveld, 2014). In the case of sturgeon migration the history of studying epiphenomenon, like the amount of fish landed by fisheries in different parts of the river, the timing and intensity of migration, the spawning periodicity, the age at first spawning, the presence and growth of early life stages (free embryos, feeding larvae, fry and fingerlings), etc., is rather common, while studying the primary causes, like the process of adherence of eggs to the substrate, the importance and influence of sedimentation and siltation on this process, the riverbed morphology and the velocity of the water above the bottom of the river in staging places d/s the spawning grounds and at preferred spawning sites, the influence of the water temperature during the embryonic development of the fertilised eggs, the presence of sympatric predatory fish threatening the fertilized eggs and early life stages, the importance of life cycle timing and characteristics of various forage animals (zooplankton and macro-zoo-benthos), etc. have been less studied.

The explanation of the mystery of the historic migration of Great sturgeons (*Huso huso*) to the Middle Danube upstream of Komarno laid in the existence, of the Great Rye Island (GroßeSchüttinsel, Csallóköz, Veľkýžitnýostrov), between the Danube, the Little Danube, and the Váh Rivers until the 16th century (Wikipedia). This was once the largest river island of Europe, 84 km long and 15 - 30 km wide, initiated during the early Miocene, 12 - 13 million years ago, on the bank of Lake Pannon, and fulfilled by the longitudinal initiation of the Danube River in the Alpine foreland, augmented by the formation of the Carpathians and the filling of the Pannonian Basin

(Neubauer et al., 2015; Winterberg & Willett, 2019). This long ParaTethys regression process leads finally to the formation in this reach of the river of about 500 islands and small islands between the river meander and side arms (Wikipedia). These were based on extensive deposits of rubble which actually formed the substrate in all the arms surrounding the islands. According to recent detailed studies in the Connecticut River (Kynard et al., 2012), rubble constitutes one of the preferred substrates for spawning of sturgeons, in case of the Danube River Great sturgeons are now known to prefer rubble substrate for spawning. Recent acoustic telemetry experiments conducted on Black rockfish (*Sebastes inermis*) demonstrated that migratory fish predominantly use the olfactory sense in their homing behavior (Matsumura et al., 2005). The homing behaviour, meaning the capacity of fish to return to their home range (birth places) for spawning, is believed to be the driving force behind the "stubborn" return of migratory sturgeons for ages to the Middle Danube River.
The intensive fishery during the Middle Ages has totally depleted the stocks, and later the first Iron Gate / Djerdap dam (rkm 943) has been blocking the access of sturgeons to the middle Danube since 1974 (Hensel & Holcik, 1997), which has made the more thorough studies of their life cycle and habitat use in the Middle Danube impossible, the status of knowledge has not changed (Guti & Gaebele, 2009). Although anthropic changes of the sediment transport in the Danube River caused by deforestation started already in the antiquity (Giosan et al., 2012), these have become the primary cause of continuous changes in spawning habitat conditions for centuries. They included the increasing sedimentation of the rubble substrate in side arms in the Great Rye Island reach of the river, even before the radical changes of the river bed caused by extensive hydro-technical regulation works in the 18th and 19th century to control catastrophic flooding as the major threat for human settlements and agricultural land use in the Pannonian plain (Hensel & Holcik, 1997; Bartosiewicz, Bonsall & Șișu, 2008). The disappearance of migratory sturgeons from the Middle Danube already before the Iron Gate damming (Hensel & Holcik, 1997) reflects the reasonable (but not always accurate) assumption that populations are likely to persist as long as habitats are maintained (Arthington et al., 2004). According to Friedrich (2018), "actions for in situ conservation target the preservation of the complete sturgeon life cycle as well as protection of its genetic diversity in its natural habitat. To meet such conservation targets, it is necessary to identify, protect, and restore the life cycle and habitats of sturgeons on the Danube River in order to prevent additional habitat alterations and to mitigate existing deficiencies. The major problem in this regard is the present lack of knowledge on habitat use, migration patterns, spawning site selection, and other autecological traits of this family. These deficiencies make further research with standardized methodologies indispensable.

This Danube migratory fish habitat manual provides to the interested public, students and a wide range of experts involved in various ongoing and future river management activities such as conservation measures, regulation and navigation projects, an useful introduction to status and habitat requirements of migratory fish in the river (Suciu, 2018). Besides an overview about the main migratory fish species with their known life cycle characteristics and the newest methods of species identification, like DNA barcoding and e-DNA, the manual also introduces the most recent methods used for identifying and describing their spawning, wintering, nursery and foraging habitats in the Danube River and its main tributaries, like acoustic telemetry for tracking their movements and behavior, sonar mapping of the river bed morphology and the substrate, use of Acoustic Doppler and electromagnetic velocity-meters to describing flow conditions, and several other approaches. The motivation for this manual is to initiate a more coherent and aligned process collecting information for a better understanding of habitats of migratory fishes in the DRB for their protection and restoration of their stocks.

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CHAPTER 2

Migratory Fish in the Danube Region

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2.1. Introduction

Fish migrate across or within environments for many reasons; the most prominent and well known motivation to migration is to spawn. Nonetheless, important migrations to wintering places or feeding grounds are not so well recognised outside the expert community. Migrations within the same environment can be either potamodromous or oceanodromous, which means migrations within the freshwater or marine environment, respectively. When the environment is changed from marine to freshwater and vice versa migrations are amphidromous. Here, anadromous migrations mean from marine to freshwater (and mostly back to the sea) and catadromous migrations from freshwater to the sea. The reason for all the abovementioned migrations is spawning. Repeated migrations for reasons other than spawning (e.g. feeding) between marine and freshwater environments is called amphidromy (Bone & Moore, 2008; Morais & Daverat, 2016). Danube migratory fish species can be divided into two groups, the anadromous and potamodromous ones. The aim of this chapter is to provide distribution, description and basic information about ecology, reproductive biology and threats for all anadromous species occurring in the Danube River Basin and for the most important potamodromous species in the Danube River Basin.
Sturgeons except for the sterlet (*Acipenser ruthenus*) and ship sturgeon (*Acipenser nudiventris*), shads (*Alosa spp.*) and Black Sea trout (*Salmo labrax)* are the anadromous fish species occurring in the Danube Basin. All of them migrated to the freshwaters several hundreds km to > 2000 km in the case of sturgeons. Some of them migrate to the freshwaters during autumn - overwinter there (autumn races of Sturgeons), early spring (spring races of Sturgeons) or later in the spring (Shads). Black Sea trout migration timing is unknown in the Danube Basin. Larvae and juveniles of Shads immediately migrate to the sea, while sturgeon juveniles stay in the freshwater environment a few months, Black Sea trout probably stay more than 1 year, but no more than 2 years.
Common carp (*Cyprinus carpio)*, barbel (*Barbus barbus*), nase (Chondrostoma nasus*)*, vimba bream (*Vimba vimba*), asp (*Aspius aspius)*, cactus roach (*Rutilus virgo*) and Danube salmon *(Hucho hucho*)are the selected potamodromous species. These species migrate for shorter distances compared to anadromous species up to a few hundreds km. Here, sterlet and the vimba bream are medium distance migratory species, while the rest are a short distance migratory species. All of them migrate for spawning in spring

starting with Danube salmon at the beginning of March followed by nase, common carp and barbel closes the spawning season in June and July.
Fishes can be identified by different descriptive characteristics. The very good description of what can be examined is provided by Kotellat and Freyhof (2007: 15-21). While examining dead specimens or when working with live fish in the field, good photos are useful for identification of species. Basic descriptive characteristics of sturgeons (Figure 1) using following abbreviations, as modified by Reinartz (2002) are as follow:
Au - unbranched rays [spinous rays, spines] of anal fin,
Du - unbranched rays [spinous rays, spines] of dorsal fin,
SD - number of dorsal scutes,
SL - number of lateral scutes,
SV - number of ventral scutes,
Sp. br. - branchial spines (gill rakers),
Tl - total length,
lc - length of head,
Fu - fulcrae.
Basic descriptive characteristics for rest of the species are as follow:
D - dorsal fin
A - anal fin
C - caudal fin
P - pelvic fin
V - ventral fin

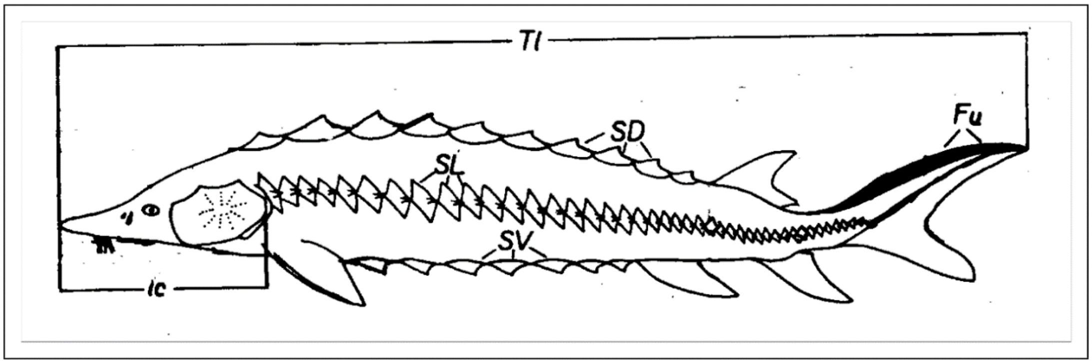


Figure 1 Descriptive characteristics in Acipenseridae used in the text. (from Reinartz, 2002, modified from Holčik, 1989)

Fins are supported by rays that are bony and soft in the rest of selected species. Bony rays are unbranched and can be serrated posteriori. The number of bony rays is referred to by Roman numerals. Soft rays are branched and their number is referred to by Arabic numerals.

2.2. Beluga - *Huso huso* (Linnaeus, 1758)

2.2.1. Historical and current distribution

Beluga was distributed in the Caspian, Black, Azov and Adriatic Sea basins. Historically beluga migrated for spawning to the MDR and sometimes to the UDR. They used to enter for spawning in many of the large MDR tributaries (Sava, Tisza, Drava, Vah and Morava), during Medieval times reaching even as far u/s as Regensburg (rkm 2380) (Hensel & Holčik, 1997).

Today beluga spawning in the DR live as juveniles and adults in the NW Black Sea (Suciu et al., 2015) and adults still migrate to the LDR until the Iron Gate / Djerdap 2 dam, at rkm 863, which do not allow their access upstream. We don't know if the Jiu River, flowing into the LDR at rkm 691.5, was historically known as spawning habitat for the beluga but in 2001 The capture of two large adults (male and female) in the Jiu River in 2001 d/s the Işalniţa dam (rkm 95) raise question if Jiu River was used for spawning (Suciu & Paraschiv, 2016).

2.2.2. Species description

Characteristics: Du 48-81, Au 22-41, SD 9-17, SL 37-53, SV 7-14, Sp. br. 17-36

As a general remark, beluga can be easily distinguished from all other sturgeon species in the DRB by their large crescent shape mouth (Figure 2 and Figure 3). YOY have razor-sharp apparent bony scutes while in adult specimens these are almost entirely absorbed in the tegument. Apart from all other DR species of sturgeons, the bony tissue of the scutes in adult beluga is almost paper-thin and cannot provide a reliable support for external tags.

Figure 2 Lateral and ventral view of the head of adult beluga (from Oţel 2007)

Figure 3 Ventral and lateral view of YOY beluga (photo credit: Marian Paraschiv / DDNI Tulcea)

During long-term (2000 - 2019) monitoring of recruitment of sturgeons in the LDR two distinct colorations of the snout/rostrum have been recorded in YOY of beluga, dark and light grey (Figure 4)

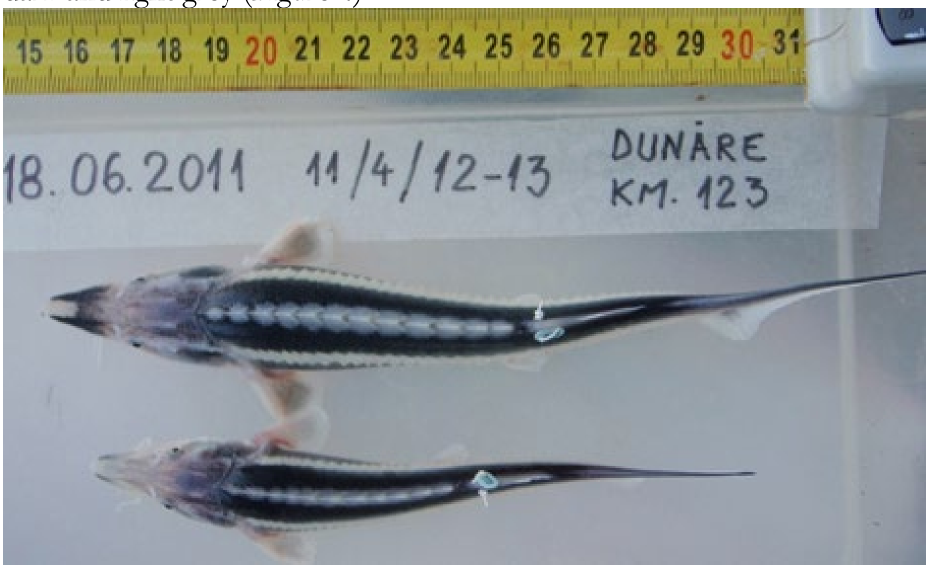


Figure 4 Distinct coloration patterns of the heads of YOY beluga captured in the LDR at rkm 123(photo credit Marian Paraschiv / DDNI Tulcea)

Pioneering hysto–morphological study by Antoniu-Murgoci (1937) has found out that the DR beluga don't have laterally compressed barbels but they are formed from a support axis and a membrane facing the mouth of the fish (Figure 5).

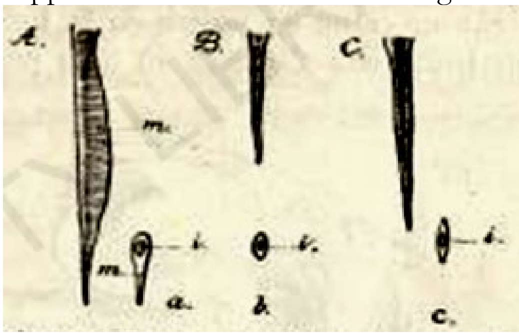
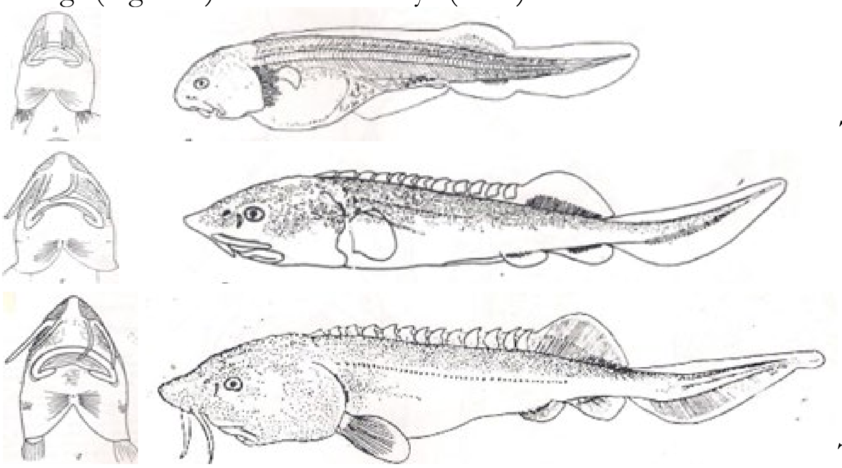


Figure 5 A- Lateral view of barbel of beluga; a- cross section of barbel; m- membrane facing the mouth of the fish; i – support axis; B, b – Acipenser stellatus id.; C, c – A. gueldenstaedtii (a variety)(from Antoniu Murgoci 1937)

2.2.3. Species identification

Late larvae fry and fingerlings of sturgeons can be identified morphologically using drawings (Figure 6) from Koblitskaya (1981).



A TL = 17.2 mm

B TL= 35.4 mm

C TL = 53.5 mm

Figure 6 Drawings of lateral views of the body and ventral views of the heads of beluga late larva (A), fry (B), and fingerling (C) (adapted from Koblitskaya 1981)

Very useful for identification of sturgeon species in trade is the CITES identification guide by Vecsei et al. (2001).

Molecular biological methods allow more precise identification of species of origin for caviar using PCR-RFLP of the mtDNA region *cytochrome b* cut with restriction enzymes (Ludwig, 2008), or using a combination of three successive statistical analyses on nuclear microsatellites (Dudu et al., 2011). As an example we are presenting the identification of species of the maternal parent in five larvae of sturgeons captured in the LDR d/s the spawning sites at rkm 100.5 (fish larva 18 /

Fig. 2.2.6) and rkm 311 (fish larvae 13, 16, 17 and 18 / Figure 7), using restriction patterns of the mtDNA cytochrome b region cut with restriction enzyme Mse I.

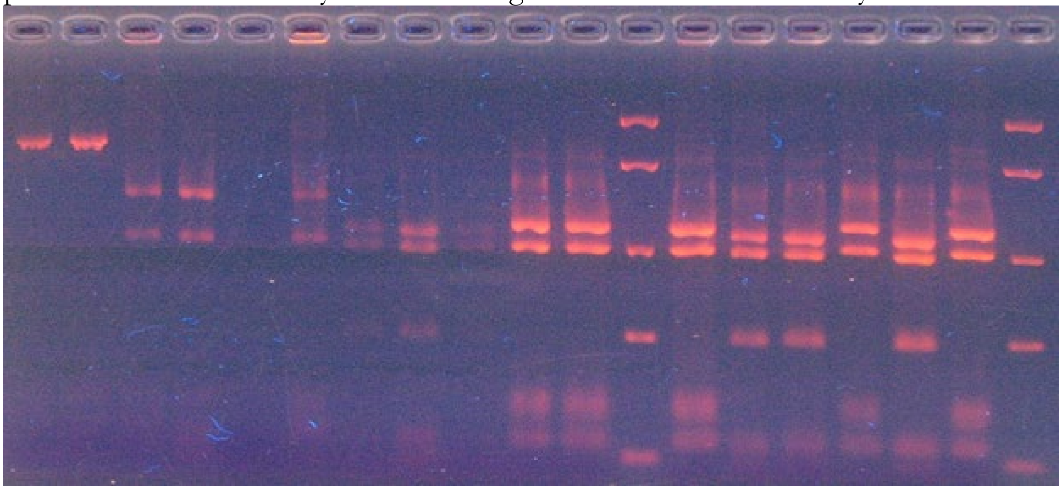


Figure 7 Restriction pattern of reference sturgeon species and larvae using restriction enzyme Mse I, based on Ludwig (2008). Lanes 12 and 19 are size standards, lanes 1 and 2 are reference adults of A. stellatus, lanes 3, 4 and 6 are reference adults of A. guel lanes 13, 16 and 18 are larvae of H. huso, lanes 14, 15 and 17 are larvae of A. ruthenus (fromSuciuet al.,2008a).

More recently, beluga larvae can be identified using the DNA barcoding method based on sequencing of the Cytochrome Oxidase subunit I gene amplified from DNA samples extracted from mince fin clips or the entire larva using the primer pair LCO1490 and HCO2198 (Ghadirnejad et al., 2009).

2.2.4. Ecology

With their historically 2,380 km long recorded migration route, beluga were the longest migratory anadromous fish species in the DR. Even 40 years after the first damming at Iron Gate, five adult beluga carrying an acoustic transmitter were recorded d/s the Iron Gate 2 dam (rkm 860) during 2014 – 2016. Three males arrived there during April and two during November – December (Suciu et al., 2016; Honț et al., 2018). The recorded ground speed of one of the males while swimming u/s, for 18 days over 747 km of the LDR was 41.5 km/day, and while searching / waiting locally over 23 km (from rkm 847 to rkm 860) the recorded ground speed was only 7.7 km/day (Suciu et al., 2016).
The persistence of historically known spring and winter races of beluga (Heckel & Kner, 1858; Herman, 1887; Antipa, 1909) in the LDR was recently reconfirmed by tracking adults implanted with acoustic transmitters (Honț et al., 2019). Spring migration starts in January or February, as soon as the ice on the river starts melting or when water temperatures surpass 4° C or the water level rises very fast in the event

of sudden breaks of naturally occurring ice jams / ice dams. Winter migration was recorded starting in October – November (Honţ et al., 2019).

During winter migration beluga stop for wintering in deep holes, when water temperatures decrease below 4° C. Acoustic telemetry enabled detection of the location of one wintering hole for beluga on the Bala branch (rkm 7.7) and one on the Borcea branch (rkm 49) (Suciuet al., 2012).

By observing the behaviour of early life stages (ELS) it was found that depending on the water temperature, post-embryonic development to external feeding in beluga lasts about 8 – 13 days (Ivanov, 1982). By ex situ experiments in artificial oval flumes it was possible to study the behaviour of drifting larvae of DR beluga, finding out that these start their pelagic drifting already as free embryos on day four after hatching, start their active external feeding with small zooplankton on day six after hatching and stop drifting d/s and swim to the ground to feed on small macroinvertebrates (worms, insect larvae and crustaceans) (Suciu, Paraschiv & Iani, 2008b).

2.2.5. Reproductive biology

A major progress in understanding reproductive biology of the beluga in the LDR was achieved by finding out that spawning happens always the day after the spring peak(s) of the water level / discharge, at water temperatures above 6 °C (Suciu et al., 2005). This enabled to shorten / optimize the fishing for monitoring recruitment of beluga from spawning in the LDR (Suciu et al., 2013). Juvenile production index charts updated annually since year 2000 (Fig. 2.2.7) show the variable recruitment, with still frequent weak year classes caused by extremely intensive / irrational fishery during 1990 – 2000 (Năvodaru, Staraș & Banks, 1999).

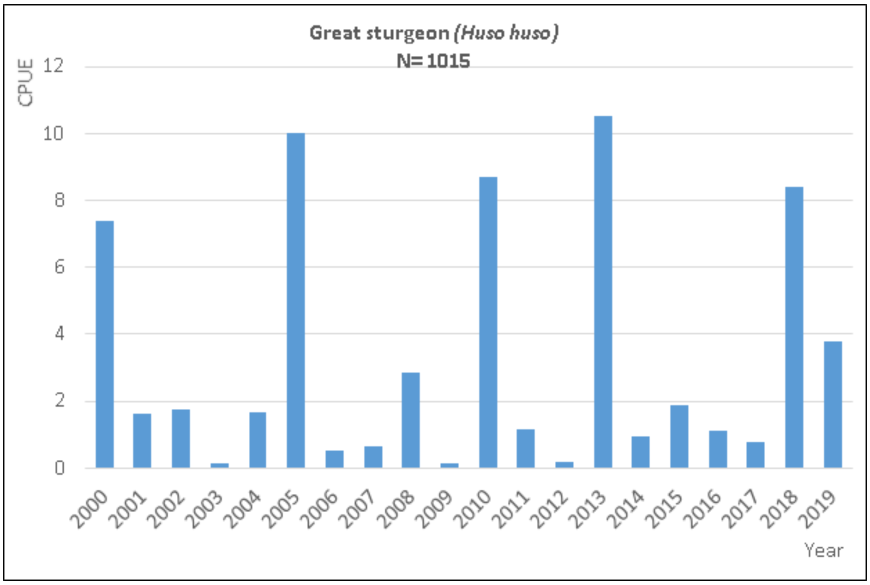


Figure 8 Juvenile production index chart showing the estimated abundance of YOY beluga at the nursery site of LDR km 123. CPUE – Average number of YOY

captured in one netting / 850 m. Data for 2018 have been corrected after Ionescu, 2018 (personal communication)

2.2.6. Threats and conservation status

DR beluga is assessed as critically endangered (Gessner, Chebanov & Freyhof 2010). They are considered extinct in the MDR and the UDR (Friedrich 2019).
Despite declaration in May 2006 of an unilateral 10 year moratorium on commercial catches of beluga by Romania and adopted in the next year also by the other range states (Bulgaria, Serbia and Ukraine), which was prolonged in 2016 for another 5 years, the DR population is threatened by illegal fishing, pollutants, bioaccumulation, habitat loss and degradation by dredging for gravel and sand, navigation rehabilitation projects, and disruption of river continuum by damming (Vecsei et al., 2002; Vassilev & Pehlivanov, 2003; Lenhardt et al., 2006; Onără et al., 2013; Suciu & Guti, 2012).

2.2.7. Missing/unknown data

The ecology of beluga in the MDR: location of spawning grounds, wintering sites, nursery sites, etc.

- If beluga adapted to spawn in the vicinity if the Iron Gate 2 dam.
- The situation of the relict part of the population with individuals born before damming the LDR at Iron Gates (1974), still sometimes arriving d/s the dam.
- If beluga has homing behaviour.

2.3. Russian sturgeon - *Acipenser gueldenstaedtii* (Brandt & Ratzeburg, 1833)

2.3.1. Historical and current distribution

Russian sturgeon was distributed in the Black Sea, Sea of Azov and the Caspian Sea basins. Historically, in the Danube River, Russian sturgeon regularly ascended to Bratislava (Heckel &Kner, 1858; Kornhuber, 1863, 1901; Herman, 1887; Ortvay, 1902 in Holčik, 1989), and rarely as far as Vienna (Heckel & Kner, 1858 in Holčik, 1989) and Regensburg (Siebold, 1883 in Holčik, 1989). The distribution area in the Black Sea is along the coast and in the Danube up to the Iron Gates. In the past, Russian sturgeon was also reported in the floodplain meadows of the Danube and in the lower parts of the Prut, Siret, Olt, Jiu, Someş and Mureş rivers (Oţel, 2007). Recently, the spawning grounds of the Russian sturgeon have been considerably reduced in size, and their migration routes have been blocked by dams, which have been built along almost all European rivers. The spring race does not travel very far into the rivers to spawn, generally only 100 to 300 km from the mouth (Holčik, 1989).

2.3.2. Species description

Characteristics: *Du* 27-51, *Au* 18-33, *SD* 8-18, *SL* 24-50, *SV* 6-13, *Sp. br.* 15-31

In the Black Sea, specimens with a length of up to 236 cm and 115 kg weight have been documented. There are also reports that in the Black Sea and the Danube River, Russian sturgeons may reach a length of 4 m (Reinartz, 2002).
There are 20 - 24 gill spines as well as mesocentric dorsal shields (Figure 9) (Oțel, 2007). The body is elongate and spindle shaped. Its height averages 12 to 14 % of *Tl*. The head length is 17 to 19 % of *Tl*. The snout is short, blunt, and somewhat rounded. Its length in specimens from the Kura River ranges from 4 to 6.5 % of *Tl*, while in specimens from the Volga and Ural Rivers it averages 5.2 % of *Tl*. The barbels are attached closer to the tip of the snout than to the mouth. The mouth is transverse, protrusive, and comparatively small. The lower lip is divided in the middle. The coloration varies from greyish black, dirty green or dark green dorsally. Laterally, it is usually greyish brown, and, ventrally, grey or rarely a lemon yellow colour. The juveniles are blue dorsally and white ventrally. Russian sturgeondoes not display sexual dimorphism (Reinartz, 2002).

Figure 9 Lateral, dorsal and ventral view of the adult Russian sturgeon (Otel 2007)

2.3.3. Species identification

The Russian sturgeon differs from the stellate sturgeon (*Acipenser stellatus*), the Atlantic or Baltic sturgeon (*Acipenser sturio*), and the Persian sturgeon (*Acipenser persicus*) in having a considerably shorter blunt snout. It usually has less than 30 gill rakers (less than the number in the Adriatic sturgeon, *A. naccarii*), and can be distinguished from the Siberian sturgeon (*Acipenser baeri*) by its simple gill rakers, which are not fan shaped; from the ship sturgeon (*Acipenser nudiventris*) by its divided lower lip; and from the sterlet (*Acipenser ruthenus*) by the lower number of scutes (less than 50) in its lateral rows and its unfimbriated barbels.

2.3.4. Ecology

In the sea, the Russian sturgeon inhabits shallow waters on the continental shelf, staying mainly in brackish water, with large concentrations of invertebrates, mainly molluscs, and small benthic fishes (mainly gobiids) (Reinartz, 2002). According to the season, these sturgeons remain at depths from 2 – 100 m or sometimes deeper (Holčik, 1989). The Russian sturgeon is a euryhaline fish that spends its life moving back and forth between freshwater and marine regions, where the salinity is 18 ‰. It requires high oxygen concentrations in the water: at least 6 or 7 mg l^{-1}. However, the sturgeon larvae are not very sensitive to the oxygen content of water. The minimum oxygen concentration for these larvae at 20 °C is 1.56 mg l^{-1} (Reinartz, 2002). In the Danube River the young sturgeons remain in deep water for a long time (Leonte, 1956, 1959; Banarescu, 1964 in Holčik 1989).

2.3.5. Reproductive biology

The maximum age reached by Russian sturgeons from the Black Sea is 33 years in the Danube region and 37 years in the Dnieper region (Reinartz, 2002). Sexual maturity is reached at 8-12 years in males and 13-15 years in females, at a body length of over 1 m (Oțel, 2007). In the Danube River the spawning sites are distributed throughout very large area, and the Russian sturgeon run continues for a long time. Spawning begins at a temperature of 8 °C, (Ambroz, 1964 in Holčik, 1989). For spawning it migrates into the river, sometimes at great distances from the mouths. Along the Danube, in the past, specimens were reported as far as Vienna. Currently, they can't cross the Iron Gates dams. As with other migratory sea sturgeons, there are two periods of migration: some spawners migrate to the Danube from the sea in February-May, with the highest intensity in April and spawning in that year, and another part begins migration from August and takes place until November, with maximum intensity in October. The latter overwinter in the Danube River and spawn in the following year (Oțel, 2007). On the other hand, Reinartz (2002) reported a single sturgeon spawning run in the Lower Danube River, which reaches its maximum intensity from July to September. After spawning, adult specimens return into the sea together with the young at the age of 2-3 months which are in size approx. 30 cm long. Some of the young can live in the lower part of the river for another 1-2 years. The food consists of various benthic invertebrates (crustaceans, molluscs, insect larvae), and adults also eat fish. The growth rate is as follows: 1 year - 31 cm, 2 years - 51 cm and 2 kg, 4 years - 77 cm and 4 kg, 6 years - 91 cm and 6.5 kg, 10 years -114 cm and 12 kg, 17 years - 160 cm and 31.5 kg (Otel, 2007).

2.3.6. Threats and conservation status

Russian sturgeon populations have been driven almost to the brink of extinction until the declaration of total ban for all sturgeon species, due to illegal catch, overfishing, breaking up of the migratory routes and pollution. Russian sturgeon is critically endangered in the Danube River Basin (Hensel & Holčik, 1997) because of late maturation, long reproductive cycles, and their long migration routes to spawning grounds disrupted by dams (Iani et al., 2019).

According to the IUCN Red List 2010 the Black Sea stock of Russian sturgeon is rated as critically endangered (CR) (Gessner, 2010).

In the Danube River the first sturgeon stocking attempts were made in the 1990s, by both Romania and Bulgaria, using the Soviet model (Bacalbaşa-Dobrovici & Patriche, 1999 in Iani 2019).

The current state of the population and locations of breeding sites is not exactly known. Young of the year (YOY) recruitment is an accurate indicator of sturgeon species' active spawning and abundance in the Danube River. Since 2000, sturgeon YOY recruitment has been monitored annually using constant parameters, in terms of fishing tools and river area. The YOY presence and abundance was assessed at rkm 123 on the Romanian side of the river downstream of Reni (UA). The fishing site is actually a feeding ground, where young sturgeons stop to feed on benthic fauna during their migration to the sea. Following the evaluation of the natural recruitment which Sturgeon Research Group within DDNI does every year, no catches have been recorded for this species since 2009. More research is needed to effectively carry out conservation measures for this species.

2.3.7. Missing/unknown data

- The ecology of Russian sturgeon in the Danube River: location of spawning grounds, wintering sites, nursery sites, etc.
- If Russian sturgeon adapted to spawn in the vicinity of the Iron Gate 2 dam?
- If Russian sturgeon have homing behaviour?

2.4. Sterlet - *Acipenser ruthenus* (Linnaeus, 1758)

2.4.1. Historical and current distribution

The sterlet historically inhabited rivers flowing into the Caspian, Black, Azov, Baltic, White, Barents and Kara Seas (Holčík, 1989; Reinartz, 2002). It was considered most distributed along the Volga River, where it is encountered through nearly the entire course, including the reservoirs and many larger tributaries (Holčík, 1989). In the Danube River, this species was regularly sighted near Passau, but rare sightings have been reported as far as Regensburg or Ulm (Bloesch et al., 2005; Reinartz, 2002). Numerous sightings were from various Danube tributaries such as Sava, Alt, Tisza, Drava, Mura, Vah, Morava, Inn, Isar, and other main tributaries in the Black Sea range (T. Friedrich et al., 2018; Holčík, 1989; Reinartz, 2002). Recently, populations have undergone rather drastic decline and distribution of this species is limited. One surviving population has been reported in the Upper Danube (Friedrich et al., 2019). Sterlet is also known to be present in the Middle and Lower Danube River. Another isolated population was reported in the Tisza River, and some other tributaries.

Dniester, Dnjeper, Prut, Mureș and Don rivers currently have naturally reproducing populations as well (Friedrich et al., 2018; Banarescu, 1994).

2.4.2. Species description

Characteristics: *Du* 32 - 49, *Au* 16 - 34, *SD* 11 - 18, *SL* 56 - 71, *SV* 10 - 20, *Sp. br.* 15 - 31, *Fu* 25 - 45

Sterlet can grow up to 125 cm and weight as much as 16 kg (Berg, 1964). However, this information is most probably true for former anadromous populations. Potamodromous specimens usually do not grow beyond 100 cm and 6 - 6.5 kg (Reinartz, 2002). There are, however, recent reports mentioning heavier fish (92 cm, 8 kg) in the Middle Danube River (Kubala et al., 2019). The body is elongated with greyish – dark brown dorsum and yellowish white belly. The greatest body height is 5.9 % to 16.6 % TL and the length of the head equals 14.6 % to 30.5 % TL. The coloration can vary depending on the conditions (Holčík, 1989). Snout length is highly variable and is considered to be the least constant characteristic for this species (Reinartz, 2002). Furthermore, sterlet can be found in two forms with both blunt and pointed snout. The ones with blunt snout were often considered as winter form and the form with pointed snout is usually considered as spring form (Reinartz, 2002). Mouth is small, transverse and barbels are fimbriated, situated closer to the mouth than to the tip of the snout. Scutes are small, usually lighter colour than the body.

2.4.3. Species identification

This species can be easily distinguished from ship sturgeon (*Acipenser nudiventris)* by lower lip, which is clearly split in the middle. From the other native sturgeon species, the sterlet can be distinguished by the number of lateral scutes being generally more than 50 (Holčík, 1989). It can be distinguished from the non-native Siberian sturgeon *(Acipenser baerii*) by white seams along all the fins.

2.4.4. Ecology

The sterlet inhabits lowland and submountain zones of rivers, where it usually stays in the current in deep sections of riverbed over stony, gravelly or sandy bottoms (Reinartz, 2002). During its life, it utilises a variety of habitats (feeding, spawning and wintering sites) that are essential for completion of its life cycle. According to Holčík 1989, the location of overwintering sites is believed to be in close vicinity of the spawning site. Recent telemetry study showed that there might be home – range behaviour present in sterlet (Kubala et al., 2019). It usually utilises spring floods for its migrations, however, it does not enter waters with high salinity. Sterlet can be sometimes found in the brackish water regions, but never descends to depths below 8 m (Reinartz, 2002). During spring floods, adults are known to migrate several weeks up the stream until they reach spawning grounds. After spawning, spent adults move downstream to areas where they intensively feed on molluscs or macroinvertebrates (Holčík, 1989). Like other sturgeons, sterlet prefers well oxygenated water (Reinartz, 2002). Oxygen concentration below 7 – 7.5 mg/l at 0.2 – 0.3 °C results in accelerated respiration of sterlet and 3.5 mg/l is their tolerance threshold (Holčík, 1989). Sterlet has the shortest life span from genus *Acipenser*. It usually lives up to 22 – 24 years and females are reported to live considerably longer than males (Reinartz, 2002).

2.4.5. Reproductive biology

The sterlet matures relatively early when compared to other sturgeons (Reinartz, 2002). According to Janković (1958) in Holčík 1989, it takes 3 – 5 years for males and 4 – 7 years for females to reach sexual maturity in the Middle Danube River. The absolute fecundity of sterlet from the Middle Danube ranges from 7,000 to 108,000 eggs in individuals from 52.2 – 77 cm *Tl* (Reinartz, 2002). Migration activity of sterlet begins when the temperature reaches 10 – 12 °C (Kalmykov et al., 2010; Reinartz, 2002) and ceases when temperature is above 22 °C (Kubala et al., 2019). Optimal temperatures for reproduction are within the range of 12 – 17 °C and spawning is discontinued if temperature drops below 9 °C or rises above 20 – 21 °C (Reinartz, 2002). Sterlet usually spawns on pebbles and eggs are laid into crevices in-between. Water velocity at spawning ranges from 1.5 to 5 m/s and oxygen concentration ranges between 6.86 – 8.31 mg/l (Shmidtov, 1939; Iogazen 1946; Khokhlova, 1955 in Holčík 1989). After the spawning, females leave the spawning grounds while males remain to participate in fertilization of eggs (Reinartz, 2002). The average weight of Danube sterlet at hatching is 0.65 g. After one month, their weight can get up to 6.8 g (Krupka et al., 2000).

2.4.6. Threats and conservation status

The sterlet is the last remaining sturgeon species that can be considered widely distributed through the Danube River basin. However, the stocks of sterlet and its status varies across countries. This is most likely because monitoring actions and restocking programmes are carried out on different scales across different countries. Sterlet is currently being listed as "vulnerable" by IUCN (Gessner et al., 2010) and fishing bans / restrictions are only enforced locally (Friedrich et al., 2018). The occurrence of sterlet in Germany and Austria is considered dependent on restocking, except for one population between Jochenstein and Aschach (Friedrich, 2013). The population in Hungary and Serbia, according to available information, can be described as unbalanced (Guti, 2006; Lenhardt et al., 2015). Moreover, there is no available information about location of spawning, wintering and feeding habitats.

2.4.7. Missing / unknown data

- Presence of homing behaviour
- Locations of spawning, wintering and feeding sites along Middle and Lower Danube River.
- Further investigation of migration patterns of sterlet sturgeons to identify cues that trigger migrations.

2.5. Stellate sturgeon - *Acipenser stellatus* (Pallas, 1771)

2.5.1. Historical and current distribution

Stellate sturgeon inhabits the Caspian, Azov, Aegean and Black Sea from where it enters rivers including the Danube River. It was always rare in the Middle and Upper Danube, only occasionally migrating upstream as far as Komárno, Bratislava, Austria or even Germany. In the past, during its spawning migrations stellate sturgeon also entered tributaries of the Lower Danube River, such as the Prut, Siret, Olt and Jiul Rivers. In the Middle Danube it migrated into the Tisza River (up to Tokaj) and the lower courses of its tributaries, the Maros and Körös Rivers, as well as the mouth of the Zagyva River, the lower courses of the Drava and Sava Rivers and the mouth of the Morava River. The species was considered rare in the middle Danube, but after the construction of the Iron Gate I and II hydropower stations, it was extirpated from both the upper and middle Danube (Hensel, 1997). As a result of river regulation and damming, the stocks of stellate sturgeon in the Black Sea watersheds have decreased significantly. The extent of spawning grounds has been reduced considerably and migration routes have been changed. Nowadays, most stellate sturgeon migrate in the Danube River only as far as the Iron Gates dams at river km 863 (Holčik, 1989). Nowadays the stellate sturgeon can be found in the Danube River downstream of Calarasi on Borcea branch but there were also recorded specimens in the Iron Gate II dam area. However, catches have dramatically declined after the damming of the lower Danube by the Iron Gate I and II hydropower stations (Bacalbasa-Dobrovici, 1997). It can also be found in the Danube River tributaries as the Prut River and the Siret River (Anon 2000).

2.5.2. Species description

Characteristics: Du 40 - 54, Au 22 - 35, SD 9 - 16, SL 26 - 43, SV 9 - 14, Sp. br. 24 - 29

Specific for the stellate sturgeon is the long and narrow snout that is dorsoventrally compressed and has a length that is varying from 59 to 65 % of the length of the head (Figure 10). This snout is characteristic for the stellate sturgeon which makes the species easily distinguished from all other members of the genus *Acipenser*. The body is covered with star-shaped plates between the rows of bonny shields. The barbels are short with the mouth lower lip interrupted in the middle and the isthmus attached to the gill membranes (Reinartz, 2002; Oțel, 2007).


M.N. 3.07.14
14/8/1/10
03/07/2014 06:57


M.N. 3.07.14
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03/07/2014 06:57

Figure 10 : Stellate stugeon (Acipenser stellatus)

2.5.3. Species identification

In case of stellate sturgeon the characteristics that distinguish this hybrid from the stellate sturgeon include a greater number of scutes in the lateral rows and a shorter snout. Sometimes the form of the dorsal scutes and the texture of the skin between the scutes are also different, and the first scute may be the largest, as in *A.ruthenus* (Reinartz, 2002).

2.5.4. Ecology

Stellate sturgeon can grow up to more than 2 meters and may reach a maximum age of 35 years. The species reaches a total length of 2.18 m, a maximum weight of 54 kg and maximum age of 35 years (Shubina et al., 1989). The common size ranges between 1.0 - 2 m and 6 - 12 kg. The age of stellate sturgeon spawners ranges between 5 - 28 years. The average weight of females is 11 - 12 kg and 6 - 7 kg for males. Females constitute 40 – 48 % of the spawning population (Levin, 1997). It takes males and females up to 5 and 10 years, respectively, to mature. The eggs are laid on vertical clay banks or on beds of scattered stones, pebbles, and gravel mixed with shell fragments and coarse sand. The optimal spawning conditions include a high flow velocity and a clean gravel bottom. A decrease in current velocity after spawning and during the development of the eggs can lead to increased losses of embryos. In the Danube River spawning occurs from May to June at temperatures between 17 and 23 °C. It spawns in 8 – 10 m deep holes with clay, gravel substrate, or on vertical clay banks. The larvae of stellate sturgeon inhabit not only the lower and middle water layers in the rivers after hatching but also occur at the surface. They drift downstream, and, during subsequent development, their capability of active movement increases. The distribution of juveniles on the bed of the Danube is influenced by food supply, current and turbidity. Juveniles migrate downstream at depths of 4 to 6 m. The life span in the river lasts from May to October and active feeding begins when the larvae reach 18 - 20 mm.

2.5.5. Reproductive biology

Spawning migration is like with other sturgeons, stellate sturgeon enters the Danube River to spawn throughout almost the entire year, but two peak periods are evident. The run begins in March at a water temperature of 8 to 11° C, reaches its peak intensity in April, and continues through May. A second, more intense migration begins in August and lasts until October. The winter migrating form hibernates in rivers and reproduces the following year. This species prefers warmer habitats than other Danube sturgeons and its spawning runs occur at water temperatures higher than those prevailing during the migrations of the other species, thus taking place immediately after those of *Huso huso* and *A. gueldenstaedtii* (Oțel, 2007). The female spawners can carry 70,000 to 430,000 eggs, depending on their size. Unlike other sturgeon species, stellate sturgeon is found in the middle and upper water layers. This species spawns on river banks inundated by spring floods and above the stony bottom of the river bed at fast currents.

2.5.6. Threats and conservation status

Stellate sturgeon was always rare in the Middle and Upper Danube and has now been extirpated from the Middle and Upper Danube as only a few individuals succeed in passing through the ship-locks at the Iron Gate dams. The last known specimen from the Slovakian section was taken at Komarno on 20 February 1926, and the last from the Hungarian stretch was reported at Mohacs in 1965. According to the IUCN Red List (2010) stellate sturgeon is critically endangered. However, according to present observations in the Danube, it is close to extinction. The current status of the population, which has suffered severely from overfishing in the past, and the exact location of the spawning sites are not known. More research is needed to effectively conduct conservation measures for this species.

2.5.7. Missing/unknown data

The ecology of stellate sturgeon upstream rkm 375 (Calarasi) and in the MDR: location of spawning grounds, wintering sites, nursery sites, etc.

- If the stellate sturgeon adapted to spawn in the vicinity if the Iron Gate 2 dam

- The situation of the relict part of the population with individuals born before damming the LDR at Iron Gates (1974), still arriving sometimes d/s the dam
- If the stellate sturgeon migration is related to a homing behaviour

2.6. Ship sturgeon/bastard sturgeon - *Acipenser nudiventris* (Lovetsky,1828)

2.6.1. Historical and current distribution

Ship sturgeon was found in the Black, Azov, Caspian and Aral Sea and some rivers in their basins. Prior to the building of Iron Gate dam, ship sturgeon was recorded in the Lower Danube (occasionally in the Danube delta) and in the Middle Danube, upstream at Komárno and Bratislava, with only exceptional migrants to the Austrian section (Hensel & Holčik, 1997; Schmall & Friedrich, 2014). Last documented specimen in Vienna was in 1936, while reports of ship sturgeon occurrence near Regensburg are not verifiable. There are also records of occasional specimens in the Danube tributaries, Váh, Tisza, Sava and Drava rivers, also two tributaries of the Lower Danube, the Prut and Siret rivers (Hensel & Holčik, 1997; Reinartz, 2002; Simonović et al., 2005; Schmall & Friedrich, 2014). Since small ship sturgeons can easily be confused with Sterlet and large specimens with Russian sturgeon, so their differentiation among fishermen is unreliable, verification based on historical documents is difficult (Reinartz, 2002).

Nowadays, ship sturgeon occurrence is limited to the Middle Danube River Section, between the Gabcikovo and Djerdap I (at the Iron Gate) power plants. Since the beginning of the 21st century, only three catches have been scientifically confirmed (Simonović et al., 2003; Guti, 2008; Guti, 2011). Due to extreme rare catches, which assumingly originate from one very small relic population, ship sturgeon in Danube River basin is near or already extinct (Hensel & Holčik, 1997; Reinartz, 2002; Bloesch et al., 2005; Jarić et al., 2009).

2.6.2. Species description

Characteristics: Du 39 - 57, Au 23 - 37, SD 11 - 17, SL 49 - 74, SV 10 - 17, Sp. br. 24 - 42

The body is elongated and spindle shaped, with blackish or greyish green coloration of dorsum and lighter on the sides. The ventral surface is yellowish-white and the fins are greyish. The body is covered with 5 longitudinal rows of scutes: one row along the dorsal midline (dorsal scutes), on mid-lateral row on each side (lateral scutes) and one row on each side between the pectoral-fin base and the anal-fin origin (ventral scutes). This species reaches a length of 221 cm and weighs 80 kg. Mouth is fairly large and the snout is pointed, smoothly rounded and conical. In adult specimens, however, the snout will grow rounder and a bit shorter compared to younger specimens.

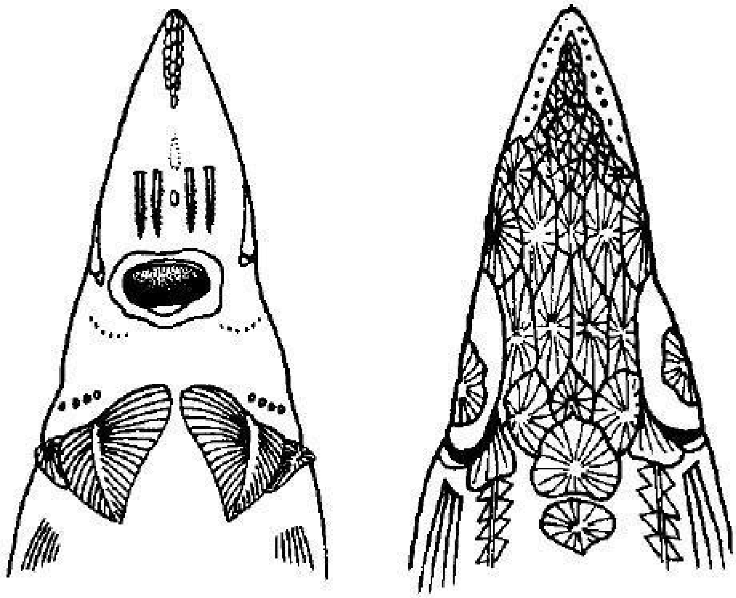

Figure 11 Ventral and dorsal view of the head of adult ship sturgeon (from Reinartz, 2002)

2.6.3 Species identification

As a general remark, ship sturgeon can easily be distinguished from all other sturgeon species in the DRB by their continuous lower lip, which is not interrupted at the centre (Figure 11). Also, the first dorsal scute is the largest. It is connected to the bone of the head and forms an obtuse angle with the profile of the head. Body depth equals 12.3 to 16.1 % of TL, while the head length is 19.1 to 22.2 % of TL. The snout is smoothly rounded and its length is 32.2 to 43.6 % of lc. The mouth is fairly large, with a continuous lower lip. Barbels are fimbriated and situated closer to the mouth than to the tip of the snout. The ventral scutes tend to disappear with age, hence the name nudiventris (Billard & Lecointre, 2000).

2.6.4. Ecology

Because of their feeding habits, the ship sturgeon remain in shallow water (above 50 m) in the sea, and they are most abundant in the vicinity of river mouths. Although the ship sturgeon forms both anadromous and resident populations, only resident form occurred in the Danube River (Hensel & Holčik, 1997). The ship sturgeon is a solitary species, and in the Danube River they feed on mayfly (Ephemeroptera) larvae, other insects, molluscs and crustaceans. During spawning migrations feeding almost ceases. Additionally, juvenile ship sturgeon tend to display more predatory habits than other sturgeons (e.g. *A. gueldenstaedtii* and *A. stellatus*), with their stomachs containing the larvae of sturgeons and other fishes. They can live over 30 years, with a maximum reported age of 36 years. Young ship sturgeons grow rather rapidly, with female growth exceeding that of the males. Populations of the ship sturgeon are characterized by a complex multi-age structure. The greatest rate of fertilization is at temperatures between 12 and 17.1 °C. Under natural conditions, ship sturgeon, produce hybrids with other sturgeon species, most frequently with *A. stellatus* (Holčik et al., 1989).

2.6.5. Reproductive biology

Data on ship sturgeon maturation in the Danube River are not available, but individuals from the Kura River attained maturity at the age of 12 to 14 years and 6 to 9 years for females and males respectively. Absolute fecundity in the Danube River is between 200,000 and 1,300,000 eggs, and the spawning takes place at water temperature between 10 and 15 °C (Holčik et al., 1989; Reinartz, 2002). Females spawn every two to three years, while males are known to spawn every one or two years (Kottelat & Freyhof, 2007). Spawning sites consist of gravelly or stony bottoms or along sections with firm, clayey sediments at water velocities between 1 and 2 m s^{-1}.

2.6.6. Threats and conservation status

DR ship sturgeon is assessed as critically endangered (Gessner, Freyhof & Kottelat, 2010). They are considered nearly extinct in the MDR and LDR, while they are extant in the UDR (Hensel & Holčik, 1997; Reinartz, 2002; Bloesch et al., 2005; Jarić et al., 2009; Sandu et al., 2013).

From the 19th century, over-fishing and river modification became the main reason for decrease in sturgeon population, while the 20th century added pollution as one of the most important factors (Lenhardt et al.2006; Lagutov et al., 2008). Additionally, a very small relic population can reduce genetic variation (through genetic drift), possibly leading to inbreeding depression and eventual extinction.

2.6.7. Missing/unknown data

- Lack of data on the Danube ship sturgeon natural mortality rates and senescence, as a prerequisite for Population viability analysis.
- Lack of data on spawning sites in the Danube River, mating and spawning habits.
- Lack of data on ecology of ship sturgeon in the Danube River: wintering sites, nursery sites, etc.

2.7. Pontic shad - *Alosa immaculata* (Bennett, 1835)

2.7.1. Historical and current distribution

Pontic shad distribution includes the entire coastline of Black and Azov Seas and it is also established in the Sea of Marmara (Erylmaz, 2001). Pontic shad enters river during spring and migrate upstream to spawn. There are two populations belonging to the eastern and western parts of the Black Sea (Navodaru & Waldman, 2003). The Eastern population migrates to Rioni, Khobi and Tzivi River to spawn (Elanidze, 1983). Pontic shad from the Azov Sea migrated to the Don River up to 900 km in the past but nowadays due to dam building it migrates only 290 km while landlocked population exist in the Kuban River (Kottelat & Freyhof, 2007). In the past Pontic shad migrated in the Danube River for about 1,600 km to Mohacs in Hungary (Kottelat & Freyhof, 2007) or as far as Budapest till rkm 1,650 (Banarescu, 1964). Nowadays due to the Iron Gate I and II dams on rkm 863 and 943, respectively, their migration was shortened but some Pontic shad could passthrough ship locks.

2.7.2. Species description

Characteristics: D III-IV 13 - 18; A II-III 16 - 20; No of vertebrae 48 - 53; No of pyloric caeca 19 - 59

Pontic shad has an elongated, herring-like body which is laterally compressed. It is dorsally dark and silvery along the ventral flanks with dark spot posterior to opercula (Figure 12) and with additional dark spots on the operculum which could disappear during upstream migration. Teeth on both jaws are well developed and strong. Size up to 350 mm standard length (Kottelat & Freyhof, 2007) and total weight is rarely more than 1 kg (Ciolac, 2004). The range of Pontic shad migrants caught in the Serbian part of the Danube River downstream of Iron Gate II was for SL from 232 - 360 mm, and total length (TL) from 268 - 375 mm and W from 126 - 452 g (Visnjic-Jeftic, 2012) while in the Bulgarian part of the Danube River (sector between Lom and Vidin) recorded SL of Pontic shad was from 154 - 370 mm (Rozdina et al., 2013). Species longevity is 5 - 7 years.

Figure 12 Adult Pontic shad caught during monitoring by drifting gillnets on the Danube River near Prahovo in 2006. (photo credit: Zeljka Visnjic-Jeftic)

2.7.3. Species identification

The three species (Pontic shad, Black Sea shad - *Alosa tanaica*, Azov shad - *Alosa maeotica*) can be difficult to distinguish where they co-occur. Gill raker counts are useful discriminatory features among adults but not for young specimens because of ontogenetic increases in numbers (Navodaru & Waldman, 2003). Among adults, Azov shad have the lowest counts of gill rakers (17 – 20; typically 17 – 19), followed by Pontic shad (34 – 69; typically 40 – 58), and Black Sea shad (67 – 91). Pontic shad gill rakers are shorter than gills (Figure 13) whereas the opposite is true for the Black Sea shad. The three species differ in typical total adult length attained, with Black Sea shad averaging 14 – 16 cm, Azov shad reaching 31 cm, and Pontic shad averaging 27 – 37 cm (Navodaru & Waldman, 2003). Teeth on palatine and vomer are well-developed in Pontic shad.

Figure 13 Gill raker of Pontic shad. (photo credit: Katarina Tosic)

2.7.4. Species ecology

Pontic shad is an anadromous migratory species, which overwinter in the Black Sea in large dispersed shoals at quite considerable depths, far from the shore, according to Bănărescu (1964) opposite the coast of Ukraine, Romania and Bulgaria, but according to Kolarov (1963) wintering shoals in front of Bulgarian coast are not found despite research efforts. Spring migrants appear first in Bulgarian southernmost coast near Resovo and then in the northern part of the coast, suggesting that wintering could happen in the south Black Sea. Adult stock live in the deep sea (50 - 100 m) and feed on fish (Navodaru, 2001). The main food of juveniles in the river is zooplankton – most important for the survival of the larvae in the first days after hatching is the presence of small rotifers *Brachionus sp.* (Kolarov, 1963). Pontic shad in the Black Sea feed mainly on small fishes (anchovies, sprats) but also consume crustaceans (Lenhardt et al., 2016; Navodaru & Waldman, 2003). Macroozobenthos (Diptera, Ephemeroptera, Plecoptera, Odonata and Planaria) were present in the diet of Pontic shad caught in the Danube River downstream of Iron Gate II which proved that Pontic shad feeds during its migration (Djikanovic et al., 2018).

2.7.5. Reproductive biology

Pontic shad migration in the Danube River begins at the end of March with the peak in April-May when water temperature reaches 9 – 17 ºC and ends in June-July (Navodaru & Waldman, 2003). Reproduction occurs in the Danube River, from rkm 180 upstream to rkm 1000 (Kolarov, 1963), with maximum intensity according to Teodorescu-Leonte et al. (1957) between Brăila and Călărași, according Kolarov (1978) between rkm 496 and rkm 743, based on the presence of females with gonads in ripe stage (VI) of maturation. First results from drifting eggs monitoring (Figure 14.) in Bulgarian – Romanian section of Danube confirms the section with most numerous drifting eggs of *Alosa sp.* is between rkm 570 and rkm 620, single eggs were registered upstream to rkm 770 (Mihov in prep.).

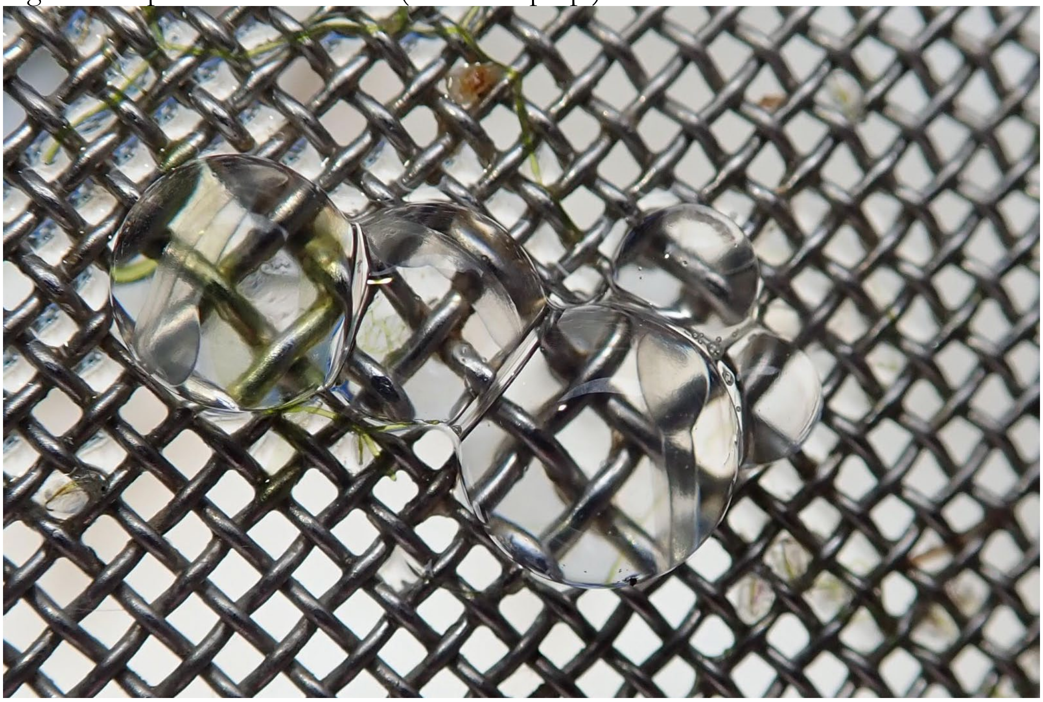

Figure 14 Ponic shad eggs with embryos before hatching Danube rkm 574 (photo credit: Stoyan Mihov / WWF BG)

Sexual maturation occurs at the age of 3 and 4 years old when shads undertake first reproduction migration, but a small proportion mature at 2 years, and approximately 10 - 17 % of shads reproduce the second time (Pavlov, 1953; Vladimirov, 1953; Teodorescu-Leonte et al., 1957, Năvodaru, 1996, 1997). Spawning of Pontic shad starts when water temperature rises above 15 ºC and is usually at 2 - 3 m depth in the main channel where current is the strongest (Kottelat & Freyhof, 2007). The fecundity estimated of the Danube River run of Pontic shad was from 13,910 till 88,983 eggs per female (Navodaru & Waldman, 2003). The egg incubation takes from 43 to 72 hours (Ciolac, 2004). Larvae and fry drift to the sea. Shad larvae which entered the floodplain and Danube Delta lakes live and feed there for several months before they move offshore to the deep sea (Figure 15) (Bănărescu, 1964; Zambriborshch & Nguen, 1973). Recently due to river embankment, most larvae drift directly to the sea and reside in the river only for 1 to 3 weeks (Navodaru, 2001a). At least some of the young are heading south and reach Cape Kaliakra already in September (Kolarov, 1963).

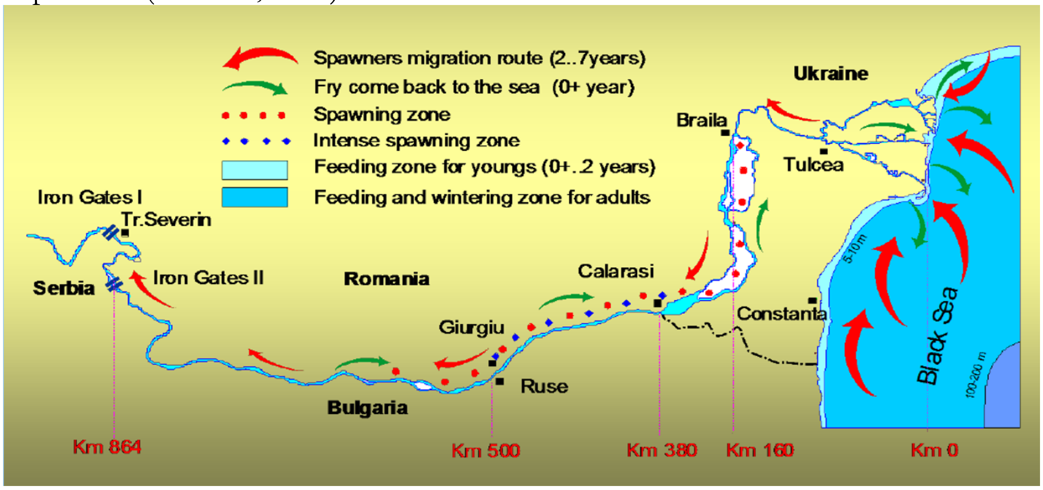

Figure 15 Migration and spawning habitats of Pontic shad in Lower Danube Region (from Ion Navodaru)

2.7.6. Threats and conservation status

The major threats are overfishing during spawning migration in the Danube River, construction of hydropower dams Iron Gates I and II which cut off Pontic shad's migration routes and embankment of the riverbanks in the Delta which don't allow larvae to enter nutrient rich floodplains and force them to drift in the sea earlier, only after 1-3 weeks spent in the river. According to IUCN, it is considered vulnerable.

2.7.7. Missing/unknown data

Pontic shad had high economic and socio-cultural value with average annual catches of 370 t in the last 25 years (Tiganov et al., 2016). In spite of that there is still missing and unknown data relating to species behaviour, spawning and recruitment, year class strength, and estimation of population dynamics (Navodaru, 2001).

Recent spawning sites and habitats are still not clearly identified, not allowing proper protection of the species.

More investigation is needed concerning migration of Pontic shad in the LDR and possibility to pass through ship locks on Iron Gate dams.

2.8. Black Sea shad - *Alosa tanaica* (Grimm, 1901)

2.8.1. Historical and current Distribution

Black Sea shad inhabits Black and Azov Sea from where it migrates into freshwater lakes and rivers to spawn and feed (Kottelat & Freyhof, 2007; Tiganov et al. 2016). In particular years Black Sea shad was numerous in the Varna Lake and the Razim-Sinoie lake complex (Teodorescu-Leonte & Munteanu, 1968). It is widespread in the Danube delta and adjacent floodplain lakes during the spring (Navodaru & Waldman, 2003). During its migration along the Danube River, Black Sea shad was recorded on rkm 375 and 496 (Kolarov, 1991; Kovachev, 1922). There is also Antipa's opinion that Black Sea shad migrated in the Danube River up to rkm 931 upstream which was confirmed by N. Bacalbasa-Dobrovici with record at rkm 864 (Navodaru & Waldman, 2003). There are new records of Black Sea shad upstream of Iron Gate II dam on 871 rkm in 2016 and 2019 (Lenhardt, unpublished data).

2.8.2. Species description

Characteristics: D III-IV 12-16; A III (IV) 16-19; No of vertebrae 48 - 50 No of pyloric caeca 26 - 50 (Svetodvidov, 2013)

Figure 16 Photo of Black Sea shad caught at rkm 871 of the Danube River in 2019. (photo credit: Gorcin Cvijanovic)

Black Sea shad is anadromous fish species and the smallest representative of three shad species in the north-western part of the Black Sea (Tiganov et al., 2016). It has an elongated laterally compressed body (Figure 16) dorsally dark with dark spot posterior to the operculum which is usually followed by 6 smaller spots (Navodaru & Waldman, 2003). Black Sea shad has the shortest head (23.7 - 25.9 % of the total length) and the biggest eyes (24.3 - 28.2 % of head length) of three shad species of north-western part of the Black Sea (Svetovidov, 2013). Teeth on palatine and vomer are poorly developed (Kottelat & Freyhof, 2007).

2.8.3. Species identification

Black Sea shad is distinguished from other shads entering fresh water from the north-western part of the Black Sea by the highest number of the gill rakers (66 - 96) which are 1.25 - 1.50 times longer than branchial filaments (Figure 17). It is also the smallest of all mentioned shads with average total length of 14 - 16 cm (Navodaru & Waldman, 2003) and size up to 20 cm of standard length (Kottelat & Freyhof, 2007).

Figure 17 Gill raker of Black Sea shad. (photo credit: Katarina Tosic)

2.8.4. Species ecology

Black Sea shad overwinter in the sea in deep layers (50 - 70 m) and move close to the shore in spring when water temperature reach 6 °C (Tiganov et al., 2016). Black Sea shad feeds mainly by plankton (Copepoda) and rarely by microflora and partly by macrophytes. In the Black Sea the main Copepoda in Black Sea shad diet are *Calanus helgolandicus*, *Acartia clause*, and *Pseudocalanus parvus*. Juvenile Black Sea shad represent a prey for pikeperch during the summer (Svetovidov, 2013). The highest amount of body fat in Black Sea shad was recorded during the autumn (33.3 - 34.6 %) while it has lower value for body fat (10.9 %) in the spring during spawning (Svetovidov, 2013).

2.8.5. Reproductive biology

Black Sea shad enters rivers when water temperature reaches about 10 °C while spawning starts at 15 °C (Kottelat & Freyhof, 2007). It is sexually mature at the age of

2 but small proportion mature at 1 year old while spawning migrants are mainly 2 (33.3 %) and 3 (61.5 %) year old with only small number of 4 year (5.2 %) old specimens (Svetovidov, 2013). Investigation in marine area of Danube Delta and along the Black Sea coast in Romania revealed that population structure comprises of 2 - 5 year old specimens both in the Danube and in the Black Sea (Tiganov et al., 2016). The fecundity of Black Sea shad ranged from 12,000 till 39,000 eggs (Svetovidov, 2013). Eggs are semipelagic and sink to bottom in non-lotic habitats (Kottelat & Freyhof, 2007). It spawns in the water column, in running Danube waters larvae drift passively to the Black Sea.

2.8.6. Threats and conservation status

Conservation status according to IUCN is Least Concern (LC). For the favourable status see Figure 18

Figure 18 Distribution and conservation status of Black Sea shad in Romania and Bulgaria. Green - favourable; orange – unfavourable (inadequate); dark gray – unknown; yellow - EU member states; (source: EIONET Portal Reporting under the Birds and the Habitats Directives Article 17)

2.8.7. Missing/unknown data

Black Sea shad is a commercial fish species which has modest economic value and protection of migrants breeding is necessary (Tiganov et al., 2016). It is important to investigate more spawning habitats and spawning migration of Black Sea shad in the Danube River.

There are statements in literature that Black Sea shad spawn a short distance from the sea (EEA 2012; Kottelat and Freyhof, 2007) but new records confirmed that it was present even on 871 rkm in the Danube River upstream of Iron Gate II dam. It is necessary to investigate if some landlocked populations of Black Sea shad exist in the Danube River as well as to provide more data about its spawning migration along the Danube River in Romania, Bulgaria and Serbia.

2.9. Black Sea trout – *Salmo labrax* (Pallas, 1814)

2.9.1. Historical and current distribution

Black Sea trout is endemic to the Pontic basin, Caspian Sea, Black Sea and Azov Sea and the rivers flowing into them. It also inhabits Lipkovska stream in upper Vardar drainage (Macedonia) and southern coast of Asia Minor, where they enter rivers over 700 km upstream from the sea (Marić et al., 2012). It was distributed in similar ecotypes as brown trout (*Salmo trutta,* Linnaeus, 1758), the anadromous, resident stream and lake ecotype. Since brown trout and Black Sea trout are not easy to distinguish morphologically nor ecologically; is exact geographic range yet to be determined with further extensive research of this species. Due to the massive stocking, the stream ecotype and the lake ecotypes were highly contaminated by the Atlantic brown trout (Kohout et al., 2012) and pure non-hybrid Black Sea trout population was not discovered until now. However, it seems that the Balkan populations in the Danube basin are less contaminated compared to the Central European populations in the Danube basin (Kohout et al., 2013). The anadromous ecotype is very rare in Europe (Freyhof, 2011) and there are just few records from the Lower Danube (Vassilev & Trichkova, 2007; Latiu et al., 2020), subadult specimens are regularly recorded in the Black Sea near the coast (Aksungur et al., 2011).

2.9.2. Species description

Salmonid species are up to 1 m in length and >16 kg in body weight (Aksungur et al., 2011), resident brook ecotypes usually not larger than 30 cm. As for all members of the family *Salmonidae*, adipose fin is present.

During the life cycle, Black Sea trout undergo well recognisable stages. Fish fry changes to parr that is typically with horizontal stripes. At the time of downstream migration to the sea, smoltification is characteristic by several morphological and physiological changes to adapt to different environment. Smolts are silver coloured with few red dots. Resident stream ecotypes are yellow-green with numerous large well visible red dots and smaller black dots. The lacustrine ecotype is less colourful, usually silver with more elongated or rectangular black spots.

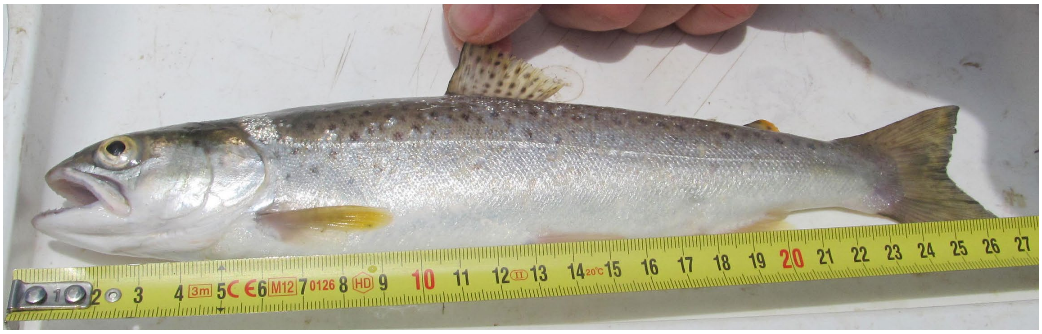

Figure 19 Smolt captured in the Danube River 9.6.2016, rkm 123.

2.9.3. Species identification

Black Sea trout is misidentified with brown trout and both species are usually still not recognised ass separate species. The main difference is the number of gill rakers on the first gill arch. While Black Sea trout has 16 - 18 (usually 18) rakers, brown trout has 13 - 18 (usually 16) rakers (Kottelat & Freyhof, 2007). Also, Black Sea trout can be distinguished from other congeners by its slightly emarginated caudal fin and yellow or orange eggs, sizing up to 5 mm (Kottelat & Freyhof, 2007).Black Sea trout (and brown trout) lack black spots on the caudal fin compared to rainbow trout - *Oncorhynchus mikkys* (Walbaum, 1792).

2.9.4. Ecology

The ecology of the resident ecotypes is probably very similar to the brown trout. Nothing is known about the ecology of anadromous ecotype in the Danube basin. Aksungur et al. (2011) suggest that Black Sea trout from Turkish rivers can tolerate higher temperature (>22 °C) compared to other salmonids. The behaviour of all ecotypes is reported as the same before smoltification. The resident ecotype lives in streams and upper parts of watercourses characterised by fast flowing and clear water and stone or gravel bottom (Kottelat & Freyhof, 2007). Resident populations can move to the sea during winter and spring. Smolts seem to migrate to the sea in spring and autumn at the age of 1+ (Aksungur et al., 2011).

Parrs spend 2 - 4 years in rivers and streams, then smoltify and migrate to sea or mature in freshwater. They spend 2 - 4 years at sea. Throughout their life in the river, individuals of both sexes retain coloration typical of the Black Sea trout. The growth of juvenile Black Sea trout during the first year of life considerably depends on temperature conditions in the water body and its food resources. The governing factor of the onset of smoltification is apparently the age rather than the sizes of fish (Nikandrov & Shindavina, 2007).

Parrs and resident adults feed on aquatic and terrestrial invertebrates, while anadromous and large lacustrine individuals prey mostly on fish and large crustaceans. Anadromous individuals feed while in rivers. Damming hinders most returning adults to reach spawning sites (Freyhof, 2011).

2.9.5. Reproductive biology

There is no detailed information about the Black Sea trout reproductive biology in the Danube. Generally, this species spawns in upper reaches with fast current, from October to January. Eggs hatch in 6 to 8 weeks (Kottelat & Freyhof, 2007). Black Sea trout from Turkish waters live in the sea for 1 - 3 years until the first migration for spawning. Fish enter the river in May-June, spawn in autumn and migrate back to the sea in November - December (Aksungur et al., 2011). Similar observations are available from Mzymta River in Russia and from various rivers of the Black Sea coast. Spawning of the Black Sea trout is largely controlled by external factors, most probably by temperature of water which can undoubtedly suppress or inhibit the maturation of individuals (Makhrov et al., 2018; Nikandrov & Shindavina, 2007).

2.9.6. Threats and conservation status

Resident ecotypes are distributed in headwaters and the pure Black Sea population was not discovered until now. All resident populations are contaminated by Atlantic lineages of brown trout.Anadromous ecotype is very rare in the Danube Basin. Sometimes, smolts also appear within the landlocked resident population, which can indicate the possibility of re-establishing anadromous populations from resident populations (Pashkov et al., 2006).

Black Sea trout is harvested for human consumption and for sport fishing. Since this species migrate upstream for reproduction, it has been impacted by the construction of dams (mostly more than three generations ago). Because of dams, most returning adults are unable to reach spawning sites and therefore to reproduce. The resident populations are less impacted by the dams (Freyhof, 2011).

Black Sea trout populations are now stabilised at a lower level and, therefore, this species does not qualify for the Threatened or Near Threatened category, and it is listed in Least Concern (LC) category on IUCN Red List (Freyhof, 2011).

2.9.7. Missing/unknown data

- Data about biology, ecology, genetic structure, habitat requirements and reproductive biology from the Danube Basin are missing.
- Further investigation of morphologic differences that can easily distinguish this species from its congeners.
- Possible natural spawning of the anadromous form in the Danube Basin.
- It is probable that the historical specimens, found in Austria, Hungary and Switzerland, represent the migratory form of the species, which is no longer present in these localities due to the river barriers present. Further genetic and taxonomic studies, together with an in-depth examination of the material currently available.

2.10. Barbel - *Barbus barbus* (Linnaeus, 1758)

2.10.1. Historical and current distribution

Barbel has a very wide distribution, inhabits most of the western, central and Eastern Europe, except northern and southern parts of the continent, also absent in Bretagne and eastern of Dnieper drainage (Kottelat & Freyhof, 2007). The species was introduced in some British and Italian rivers. Barbel mainly occurs in the barbel to grayling zone in the Danube Basin.

2.10.2. Species description

Medium to large, spindle-body fish species. It has two pairs of barbels. The back is golden-green with lighter sides, almost white in the chest area. Spots can occur on juveniles and sub-adults. The lower lobe of caudal, as well as anal fin, are orange-reddish to light brown in older specimens. It has 53 - 63 total scales on the lateral line; dorsal spines (total): 3 - 4; dorsal soft rays (total): 7 - 9; anal spines: 2 - 3; anal soft rays: 5 - 6; vertebrae: 46 - 47. The origin of pelvic fin is about below that of the dorsal; scales with free posterior part, which is pointed; scales on back with 1 - 5 well developed median longitudinal epithelial crests (Kottelat & Freyhof, 2007). Caudal fin has 19 - 20 rays (Spillman, 1961).

Figure 20 Juvenile barbel from Yantra River (Bulgaria) caught during monitoring by electrofishing.

2.10.3. Species identification

Lower lip is thick, with a median swollen pad. Dorsal fin with pointed tip, also concave posterior margin. The posterior hard ray of this fin is serrated along the entire posterior edge. There are no spots in larger specimens. There are 53 - 63 scales on the lateral line. The origin of pelvic fin lies below the origin of dorsal fin (Kottelat & Freyhof, 2007). The last unbranched dorsal ray is spinous with posterior serration. About 20 – 24 % of the last simple dorsal ray's length is flexible. It can be misidentified with small barbel species *Barbus petenyi* Heckel, 1852, *Barbus carpathicus*

Kotlík, Tsigenopoulos, Ráb & Berrebi, 2002 and *Barbus balcanicus* Kotlík, Tsigenopoulos, Ráb & Berrebi, 2002 which have longer anal fins exceeding the half distance between anal and caudal fin base and the last unbranched dorsal fin ray is soft.

Figure 21 Sympatric populations of Barbus barbus (right) and Barbus petenyi (left). Differences in caudal and dorsal fins morphology, anal fin length, head shape and coloration are visible.

Pharyngeal teeth formula: 2,3,5 - 5,3,2

2.10.4. Species ecology

Barbel is a rheophilic, lithophilous, potamodromous cyprinid. The species prefers sections with clean, cold and fast-flowing waters with a gravel substrate. It can be found in lakes. Adults often aggregate. It is most active during dusk and dawn (Kottelat & Freyhof, 2007).
Larvae and juveniles are active during both day and night. It feeds on a wide variety of benthic invertebrates, small fish and sometimes on algae. The life span of the species ranges 15 - 20 years (Bănărescu et al., 2003; Trigo et al., 2017).

2.10.5. Reproductive biology

The males reach sexual maturity at the age of 2 - 5 years, and the females at the age of 3 - 4 years (Kottelat & Freyhof, 2007). Before spawning they gather in groups and migrate upstream. Eggs are released in May-July. The fecundity of

Figure 22 Rapids in the middle flow of the Osam River (Bulgaria): feeding habitat for sub-adult Common barbel.

females ranges from 3,000 to 155,400 eggs. It attains a maximum body length of 120 cm and a weight of 12 kg (Bianco, 1998). Spawning is portional, usually at intervals of 10 - 15 days each season. Individual females spawn with several males. Males assemble at spawning grounds and follow ripe females, often with much splashing, to shallow riffles. Males may exhibit courting or sneaking tactics in spawning sites. Courting males follow females to the spawning site and, during the spawning act, one male swim head to head with the female. Sneaking males, waiting in the spawning site, then join the couple and try to fertilize eggs. Up to 130 males have been reported to be involved in a single spawning act. Females deposit 2 - 3 portions of non-sticky eggs into excavations made in the gravel. Feeding larvae drift a short distance from spawning sites to shallow shoreline habitats. Larvae and juveniles are benthic, in very shallow shoreline habitats. They leave the shores for faster-flowing waters as they grow (Kottelat & Freyhof, 2007).

2.10.6. Threats and conservation status

Conservation status according to IUCN is Least Concern (LC)
Channelization of rivers reduced fish biomass and led to the disappearance of migratory and rheophilic species (Schiemer and Waidbacher, 1992). Main threats are water pollution, dams, river regulation, overfishing, poaching. According to the Bulgarian National and Aquaculture Fishing Agency, the commercial catches of barbel between 2016 and 2018 were decreased from 6,700 to 5,046 tons, which

probably indicates population decline. During the 20th century, the threats were construction of large reservoirs and pollution, but populations are recovering in central Europe.

2.10.7. Missing/unknown data

- Migrations of Common barbel and some basic ecological aspects in the Danube Basin; abundance of older specimens, length-weight relationship, total and fishing mortality, population trends etc.
- Genetic variability in the LDR.
- Annual catches for all Danubian countries.

2.11. Nase - *Chondrostoma nasus* (Linnaeus, 1758)

2.11.1. Historical and current distribution

Native in Black, southern Baltic and southern North Sea drainages. It was introduced in some Atlantic and Adriatic Rivers (Kottelat & Freyhof, 2007). Barbel mainly occur in the barbel to grayling zone in the Danube Basin.

2.11.2. Species description

Nase has a robust body, with a grey back and silverfish sides. It has a sharp lower lip. The mouth is located under the snout; it is almost straight, vertical to the head. Fins are orange-reddish, especially near their base. It can reach 50 cm and 1.5 kg (Muus, B., & Dahlström, 1968).

Figure 23 Nase from the Vit River (Bulgaria).

2.11.3. Species identification

The mouth is straight in older specimens, the sheath on the lower lip is cornified and thick. Branched rays on anal fin $9^{1/2}$, on the anal $10\text{-}11^{1/2}$. The eye is large, about 50 – 65 % of interorbital distance. There is no dark stripe laterally on the body. There are 52 - 66 scales on the lateral line, 27 - 36 gill rakers. The fish is very easy to discriminate from other cyprinids in the Danube Basin with its typical mouth.
Pharyngeal teeth formula: 6-6
Populations from UDR show rather high levels of gene flow. This conclusion is supported by the distribution of rare alleles at several allozyme loci, which occur in similar low frequencies (Gollmann et al., 1997).

2.11.4. Species ecology

Nase is a rheophilic, lithophilous, potamodromous cyprinid. It is an indicator species for habitat quality in the lower rhithral and upper potamal zones of European river systems and one of the most vulnerable species (Nelva, 1997). It moves and feeds in groups, often formed by hundreds of specimens. Appropriate habitats show high population density locally, whereas in other nearby areas it is almost 0. Higher activity is shown about midday (MEASURES monitoring, unpublished data). The habitat use of nase was studied by Huber, Kirchhofer (1998).
Nase feed mainly on periphyton and larvae of Chironomidae (Losos et al., 1980). It can reach 46 cm and 2.3 kg at the age of 12. Von Bertalanffy growth parameters can vary according to biotic (food availability) and abiotic factors. Fast growing and slow growing groups have been established in MDR. Sexual maturity is reached rather late, after 7 - 8 years in MDR (Vater, 1997). It can live up to 20 years (Hudson et al., 2014).

2.11.5. Reproductive biology

Nase spawn from March to early May following a temperature rise to 8 - 9 °C (Philippart, 1980). The amount of eggs released from large individuals can be several times the amount of eggs small females can produce. 35,000 – 45,000 eggs/kg body weight can be expected. The eggs are hatched after about 11 days at 12 °C. (Kainz & Gollmann, 1999). Substrate composition is crucial for hatching and early development (Nagel et al., 2019). After hatching the larvae move deeper into the interstitial zone (Duerregger et al., 2018) Water velocity is also a key factor for determining the nursery habitats (Keckeis et al., 1997).

2.11.6. Threats and conservation status

The conservation status according to IUCN is Least Concern (LC).
Main threats are represented in the habitat alterations and damming, as well as pollution (Huber & Kirchhofer, 1998). Channelization of rivers reduced fish biomass and led to the disappearance of migratory and rheophilic species (Schiemer & Waidbacher, 1992). Some local populations are today extinct, and the remaining populations are generally smaller and declining in numbers (Maier et al., 1995).

Figure 24 Upper middle flow of the Vit River (Bulgaria). Spawning habitat of the nase.

Figure 25 . Migratory barrier for the nase in the upper middle flow of the Vit River (Bulgaria).

There is also overfishing and poaching. According to the Bulgarian National and Aquaculture Fishing Agency, the commercial catches of Nase between 2016 and 2018 were decreased from 1,309 to 1,203 tons, which probably indicates population decline.

2.11.6. Missing/unknown data

- Nase migrations, as well as some basic ecological aspects: abundance of older specimens, length-weight relationship, exact location of spawning grounds and time of spawning, growth, total and fishing mortality, population trends etc.
- Genetic variability
- Total annual catches for all Danubian countries.

2.12. Vimba bream - *Vimba vimba* (Linnaeus 1758)

2.12.1. Historical and current distribution

A widespread semi-anadromous species, distributed in Caspian, Black, Marmara and Baltic Sea watersheds (Sweden and Finland north to 62 - 63°N), North Sea basin from Elbe to Ems drainages. In Finland it is restricted to coastal waters. Absent in eastern Anatolia (Freyhof, 2010). It is introduced in the Rhine (Freyhof, 1999). Inhabits mailny grayling and barbel zones in the Danube Basin.

2.12.2. Species description

The mouth is small and localized subterminal behind the snout. It is a deep bluish-green on the dorsal surface and silvery on the sides. The eyes are yellow and the pectoral and pelvic fins have reddish-yellow bases. During the spawning season, males become darker with orange cheeks and belly (Kottelat & Freyhof, 2007).

Figure 26 Juvenile vimba bream from Lefedja River (Yantra tributary, Bulgaria).

Dorsal spines (total): 3 - 3; Dorsal soft rays (total): 8 - 9; Anal spines: 3 - 3; Anal soft rays: 16 -22. Caudal fin has 19 soft rays. The scales are relatively small, their number on the lateral line counts 49 - 64 (Keith & Allardi, 2001).

2.12.3. Species identification

The body of the vimba bream is not as deep as that of the Common bream. Back keel is present behind dorsal base. Scaleless dorsal groove is in front of dorsal. There are 16 to 22 branched anal rays (Kottelat & Freyhof, 2007).
Pharyngeal teeth formula: 5-5
Cytochrome b gene and its mitochondrial control region, the description of the obtained haplotypes and their comparison were studied by Buburuzan et al. (2008). Cytochrome b gene shows a rather low genetic diversity; western populations derived from an ancestral Danubian clade (Braendle et al., 2009)

2.12.4. Species ecology

Vimba bream is a riverine or semi-anadromous, batch-spawning cyprinid fish species. Some populations are anadromous, feeding in brackish waters (Braendle et al., 2009). It inhabits large and medium rivers, but also lakes and reservoirs. It is an omnivorous species, feeding on various invertebrate groups, as well as phytoplankton and detritus (Okgerman et al., 2013). In MDR the food variety of the species is narrower, which prefers gammarids, avoiding Chironomidae and Diptera (Egerić et al., 2015). Vimba bream lives up to 15 years.

Figure 27 Feeding habitat of vimba bream in the lower course of the Iskar River (Bulgaria).

2.12.5. Reproductive biology

Spawns for the first time at 3 - 5 years, during May-July when temperature rises above 15 °C. Some populations spawn earlier. Semi-anadromous populations feed in freshened parts of sea and undertake long distance spawning migrations. Lacustrine populations migrate to fast-flowing tributaries. Individual females spawn several times during a period of 2 - 3 weeks. Usually spawns for several years. After the reproduction adults return to feeding habitats (Freyhof, 2010).

2.12.6. Threats and conservation status

According to IUCN: LC
According to Bern Convention: Appendix III - Protected fauna species
Biocorridor fragmentation/barriers, other habitat modifications and altered water regimes, as well as pollution, may also have negative effects (Tambets et al., 2018; Popovic et al., 2012).
Channelization of rivers reduced fish biomass and led to the disappearance of migratory and rheophilic species (Schiemer & Waidbacher, 1992). There is also overfishing and poaching. According to the Bulgarian National Aquaculture and Fishing Agency, the commercial catches of vimba bream between 2016 and 2018 were decreased from 1,546 to 1,351 tons, which probably indicates population

decline. Some populations are threatened; it is characterized as "vulnerable" to "critically endangered" in the Czech Republic and is included in the Red Data Book of the country (Lusk et al., 2005)

2.12.7. Missing/unknown data

- Migrations of vimba bream in the Danube Basin, as well as some basic ecological aspects: abundance of older specimens, length-weight relationship, exact location of spawning grounds and time of spawning, growth, total and fishing mortality, population trends etc.
- Genetic variability
- Total annual catches for all Danubian countries.

2.13. Cactus roach – *Rutilus virgo* (Heckel, 1852)

2.13.1. Historical and current distribution

Cactus roach is distributed in the Danube basin upstream the Iron Gate. It inhabits medium size to large rivers. It is most abundant in the Sava River (Kottelat & Freyhof, 2007) and its southern tributaries. Populations in other rivers are probably underestimated. In 2012-2014, the relative density of cactus roach during sampling with large seine nets and trammel nets at the Danube near Iža and Radvaň nad Dunajom varied between 0 to 2 % per haul.

2.13.2. Species description

Cyprinid species which is similar to roach (*Rutilus rutilus,* Linnaeus 1758) with smaller head, larger snout, yellow-red eye, shiny coloration, black peritoneum and larger size exceeding 400 mm and 2 kg (Figure 28). Lateral line has 44 - 52 scales, with 7 - 10 scale rows above and 4 -6 below the lateral line. Pharyngeal teeth are in one row with formula 6 - 5 or 5 - 5 (Mišík, 1957; Talabishka et al., 2015). Young fish are with typical orange-red coloration on the bottom lobe of caudal fish (Figure 29). Adults are more intensely coloured.

2.13.3. Species identification

Cactus roach can be distinguished from roach by 44 - 52 scales in lateral line, black peritoneum and more shiny coloration compared to Roach with (37) 41 - 43 (46) scales in lateral line and light peritoneum. Cactus roach males have prominent tubercles on the head and body during the spawning season with two horizontal rows of tubercles on the head (Figure 30). Talabishka et al. (2015) identified possible mis-identification with Ide, *Leuciscus idus* (Linnaeus, 1758), where mouth of Ide is terminal, while mouth of Cactus roach is inferior and Ide has more than 56 scales in lateral line. Cactus roach was considered as a subspecies of *Rutilus pigus*, but Ketmaier et al. (2008) identified the Danubian populations as distinct species by genetic markers.

Figure 28 Large male of cactus roach during spawning (Sl about 370 mm)

Figure 29 Young cactus roach (Sl about 150 mm)

Figure 30 Head of Cactus roach during spawning with two horizontal rows of tubercles. 3. 4. 2019, the Váh River near Trenčín.

2.13.4. Ecology

Ecology of the Cactus roach is mostly unknown. It inhabits medium to large rivers near the bottom. Jurajda & Pavlov (2016) and Povž et al. (2015) suggest a shoaling behaviour as for other roach species. Most probably feeds on macroinvertebrates and fish eggs.

2.13.5. Reproductive biology

Reproductive biology of the Cactus roach is mostly unknown. The maturity is reached at the age 2 - 3 for males, females one year later (Holčík, 1995). In Slovenia, the Cactus roach spawns from March to May in shallow gravel beds with a strong flow of water when the water temperature is between 10 - 14 °C. Its spawning sites are shared with Nase, *Chondrostoma nasus* (Linnaeus, 1758), Grayling, *Thymallus thymallus* (Linnaeus, 1758) and Barbel, *Barbus barbus* (Linnaeus, 1758) (Povž et al,. 1998). The information about spawning on submerged plants (Kottelat & Freyhof, 2007) seems not to be validated.

2.13.6. Threats and conservation status

Kottelat & Freyhof (2008) listed Cactus roach as Least Concern according to IUCN. It is listed in the EU Habitats Directive. It seems that it is more common than it was reported in the past. To confirm it, more specialised research is needed.

2.13.7. Missing/unknown data

- Recent population status in all rivers.
- Genetic structure in the Danube Basin.
- Habitat requirements.
- Reproductive biology – number of eggs of Cactus roach is 6,500 - 57,000 (Talabishka et al., 2015)
- Migrations, home range – medium. distance migratory species (potamodromous) (Povž et al., 2015).

2.14. Asp – *Leuciscus aspius* (Linnaeus, 1758)

2.14.1. Historical and current distribution

Formerly it was included in the genus *Aspius,* but Perea et al. (2010) showed that it is grouped in the same lineage with genus *Leuciscus*. The Asp (*Leuciscus aspius* Linnaeus, 1758) inhabits middle and lower reaches of rivers, as well as lakes and reservoirs in Central and Eastern Europe, southern Scandinavia, Caspian and Aral Sea drainages and north-western Turkey (Kottelat & Freyhof, 2007). It is also native in the North Aegean Rivers and their main tributaries (Vassilev & Pehlivanov, 2005). It is absent in the southern part of the continent. In the past it was common in the lower courses of the southern/eastern Danubian tributaries (Bulgarian territory), now it is only occasionally registered in their estuaries. It is probably extinct in the Bulgarian Black Sea biogeographical zone (Kamchia R. watershed). It is more common in the MDB and UDB. It has been introduced in some countries of Western and southern Europe, including France (Schweyer et al., 1991) and Spain (Merciai et al., 2017).

2.14.2. Species description

Asp was a single representative of genus *Aspius* in Europe. It has a fusiform body that is long, and laterally compressed, with a long pointed snout. Most of the body has a silvery colour over most of its body, with blackish-olive or greenish-grey back. The iris is silvery, with a narrow golden circle around the pupil and a little grey pigment on the upper half. Its lips are silvery with a little grey over the upper one; however, both lips and iris are often bright red. The dorsal and caudal fins are grey and the other fins are transparent without pigment (Keith & Allardi, 2001)

Figure 31 Asp from LDR, about 50 cm length.

It is distinguishable from other European cyprinids by the angle of the jaws and with maxilla extending beyond the front margin of eye. There is a sharp scaled keel between the origins of pelvic and anal fins. Total count of scales in the lateral lines is 64 - 76. Number of soft rays in dorsal fin is 9 and 12 -15 in anal fin. Pharyngeal teeth formula in general is 5.3-3.5.

2.14.3. Species identification

It can be easily recognized morphologically even in the postlarval stage by the disposition of the angle between jaws. Hybrids are not common, although mentioned before (Kottelat & Freyhof, 2007). The electrophoretic patterns of some parvalbumins ofasp have been examined and the obtained patterns occurred to be species-specific (Bobák & Slechta, 1988).
Four microsatellites were isolated from asp roe from the catchment of Lake Malaren-Sweden (Glans, 2007).

2.14.4. Species ecology

Asp is a rheophilic, benthopelagic, potamodromous fish from the family Cyprinidae. Although asp is present in stagnant water bodies as well as in rivers, it is considered to be a rheophilic (type B) species. In the Elbe, asp seldom visited natural oxbows for wintering or feeding (Fredrich, 2003). In addition in LDR it is a common inhabitant of riparian lakes (Pehlivanov et al., 2011; Pehlivanov et al., 2017) (Schiemer & Waidbacher, 1992). It can live for more than 10 years. It matures in 3 - 5 years. It shows good swimming ability (2.1–2.4 ms^{1}, even 3 ms^{-1}) (Fredrich, 2003). The diet spectrum of the species consists exclusively of fishes, especially on Bleak (*Alburnus alburnus)*.

2.14.5. Reproductive biology

It spawns at places with strong current on sandy or pebble substrate, when water temperature rises above 8 ºC. Ray (2005) stated that the reproductive success seems to be associated with low water level and high spring temperatures, spawning lasts about two weeks and eggs are deposited to gravel or submerged plants. It can reach 4 - 5 cm during early summer and 7 - 8 cm length during first autumn indicating quick growth (MEASURES monitoring, 2019). Incubation lasts for 10 - 15 days, with fecundity from 58,000 to 500,000 eggs which are 1.6 mm in diameter (Berg et al., 1949).

2.14.6. Threats and conservation status

According to IUCN: LC

In the past, the asp was common in the lower courses of the most southern/eastern Danubian tributaries (even in the middle course of the rivers Iskar and Yantra), now only occasionally it is registered in their estuaries. It is still abundant in the MDB. Main threats for the species are the fragmentation of biocorridor/barriers (Fredrich, 2003), habitat alterations and destruction; pollution. Channelization of rivers reduced fish biomass and led to the disappearance of migratory and rheophilic species (Schiemer & Waidbacher, 1992). Overfishing and poaching also pose a threat. Janković (1967) assumed that older specimens of asp are rare in this part of the Danube due to intensive fishing pressure since the 1960s. According to the Bulgarian National Aquaculture and Fishing Agency, the commercial catches of asp between 2016 and 2018 were decreased from 1,014 to 617 tons, which probably indicates population decline.

2.14.7. Missing/unknown data

- Migrations of Asp in the Danube Basin, as well as some basic ecological aspects: abundance of older specimens, length-weight relationship, exact location of spawning grounds and time of spawning, growth, total and fishing mortality, population trends etc.
- Genetic variability
- Total annual catches for all Danubian countries.

2.15. Common carp – *Cyprinus carpio* (Linnaeus, 1758)

2.15.1. Historical and current distribution

Common carp *sensu lato* that is mostly accepted in the literature is naturally distributed in Euroasia. In recent years, subspecies are recognised as species and Common carp *sensu stricto* as the former *Cyprinus carpio carpio* was naturally distributed only in the Black, Caspian and Aral Sea basins. In the Danube, its uppermost distribution was in the Slovak-Hungarian section up to Morava (March) mouth (Balon, 1995). Later, it was introduced worldwide, mainly the East Asian strains/species. The original wild

Danubian common carp population is most probably contaminated by the domesticated carps (Kohlmann, 2015) or is probably extinct (Piria et al., 2016).

2.15.2. Species description

Cyprinid species with two pairs of barbels with body size up to 1100 mm and body mass up to 40 kg (Kottelat & Freyhof, 2007). Wild form of the Danube common carp is a powerful, elongated and torpedo-shaped animal with large, regular scales and golden (yellow-brown) color. The dorsal contours of its head and body continue in one smooth curve. The transition between the head and body dorsum is nearly straight, without the clear notch (depression) typical of domesticated or feral carps. Scales are regular and large, and their caudal edges are marked by dark pigment, giving the body a mesh-like appearance. The coloration is brown to dark brown on the dorsum, dark golden at the sides and light golden with an orange twinkle on the ventral part. The leading spines of the anal fin are yellow-orange, as is the part around the anus. Other fins are dark brown. Wild Danube common carp usually with 29 - 36 gill rakers (mean 32), (34 - 35) 36 - 39 (40) most frequently 37 - 39 scales in the lateral line and long dorsal fin with 18 - 21 (22), most frequently 19 - 20 branched rays. The body depth is 24.6 - 30.9 % (mean 27.7 %) of Sl (Balon, 1995).

Figure 32 Wild common carp from LDR (fish market, Tutrakan, Bulgaria, 2010)

Figure 33 . Carp from LDR, aquaculture form (Danube monitoring, Krivina, Bulgaria, 2014)

2.15.3. Species identification

Common carp can be misidentified with cyprinids from the genus *Carassius* which lack barbels. Asian strains/species of the genus *Cyprinus* has generally less gill rakers (24 to 31, mean 27) with small overlap. The number of scales on the lateral line, or branched rays in the dorsal fin, overlap in greater manner (Balon, 1995), so molecular markers should be used to distinguish between them. Domesticated forms inhabiting lotic environments can form low body forms. So, molecular markers should be used to distinguish between domesticated and original wild individuals.

2.15.4. Ecology

The wild Danube common carp inhabited free flowing sections of lowland or sub-mountain rivers (Balon, 1995). Preferred habitats are unknown. They are omnivorous species that feed on the bottom.

2.15.5. Reproductive biology

The wild common carp can be classified as a non-guarding, open substratum egg-scattering, obligatory plant spawner with portional spawning. Each female released two or three portions of eggs within a 10 - 14 day interval. The spawning commenced at about 18 °C when large schools of carp entered flooded grass flats within the inundation areas of the river. Meadows, freshly flooded to 25 - 50 cm, were the preferred substrate at all times. Mating groups usually consisting of one female and several males circled ferociously, often disturbing the surface or with dorsal parts out of the water. Spawning was interrupted when the water temperature fell below 17 °C. Males in the spawning stock belonged to the age groups 3 - 15, mostly in the age group 5. Females were represented by age groups 5 - 9, most of them in age group 7. It seems that domesticated individuals do not spawn together with the wild form. The outer egg envelope is adhesive and sticks to grass blades, preventing the eggs from falling into the anoxic bottom. At about 20 – 23 °C most embryos hatch in 3 days

(Balon, 1995). There is some information about the reproductive biology from the Iranian coast of the Capsian sea, but the natural wild origin of this population is not justified (Vazirzadeh & Yelghi, 2015).

2.15.6. Threats and conservation status

Wild Danube common carp was considered very rare and can be probably extinct, however possible reproductive isolation between the wild and domesticated form indicated by Balon (1995) can enable the survival.

2.15.7. Missing/unknown data

- No information about the viable wild Danube Common carp population is available.
- Habitat preferences of wild Danube Common carp are unknown.
- Information about the ecology of reproductive biology comes generally only from the population from Slovakia.

2.16. Danube salmon - *Hucho hucho* (Linnaeus. 1758)

2.16.1. Historical and current distribution

Danube salmon is an endemic species historically distributed in the Danube River Basin occurring in medium and large rivers including Danube upstream Iron Gate (Holčík, 1990). It inhabits sub-montane rivers draining the Alps, the Dinaric Alps, the Carpathians, and partly also the Bohemian-Moravian Highland, the Šumava (Bohemian Forest) Mountains and the Weinsberger Wald Hills (Holčík et al., 1988). Its range has considerably decreased (Figure 34).

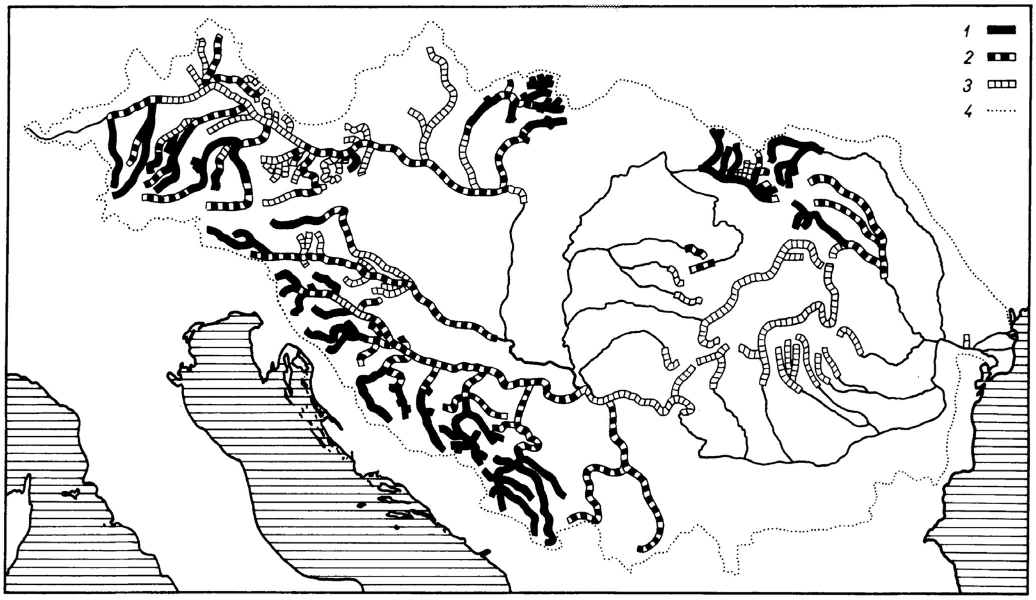

Figure 34 Historical and recent distribution of the Danube salmon. 1 - present permanent occurrence, 2 - present sporadic occurrence, 3 – historically documented past occurrence, 4 – boundary of the Danube Basin (From Holčík et al. 1988)

The species was considered at the end of 20th century as extinct in more than 39 % (4353 rkm), 27.5 % (3055 rkm) was rare and more or less common only in 33.4 % (3718 rkm) of its former range. The population is fragmented in most rivers and mostly dependent on stocking (Holčík, 1990) probably except for some Austrian rivers (Weiss & Schenekar, 2016). A total of 1842 rkm with self-sustaining populations of Huchen exists in the Balkans. These populations are found in 43 river sections in Slovenia, Croatia, Bosnia-Herzegovina, Serbia and Montenegro. For the Balkan region, by country, and counting border rivers twice, 1072 km of the total habitat is found in Bosnia-Herzegovina, 456 km in Slovenia, 391 km in Serbia, 240 km in Montenegro, and 228 km in Croatia. Of these, approximately 42 % (688 km) were considered to support populations that were stable, 22 % (354 km) increasing and 36 % (588 km) decreasing (Freyhof et al., 2015).

2.16.2. Species description

Danube salmon is a large salmonid species that can reach a maximum total length of up to 183 cm and a weight of up to 60 kg (Holčík et al., 1988) which makes it a part of freshwater megafauna of the area. It has a fusiform body with relatively large head and big mouth. As for all members of the family, adipose fin is present. Lateral line with 115 - 160 perforated scales, with 28 - 38 scale rows above and 25 - 35 rows below the lateral line. D 8 - 12, A 7 - 10. First gill arch with 10 - 14 (including rudimentary19) gill rakers (Hensel & Holčík, 1983).

Danube salmon coloration is rather variable changing with age and within populations. It has dark back varying from greenish to brownish with black spots. The reddish coloration never exceeds to the abdominal part of the body which is silvery grey. YOY fishes up to 2 months are dark, later with vertical dark bands that disappear at the age of one year. The margins of the fins are never white (Holčík et al., 1988; Kottelat & Freyhof, 2007; Povž et al., 2015) (see Figure 35, Figure 36 and Figure 37).

Figure 35 Young Danube salmon from the Hron River near Brezno (SL about 100 mm)

Figure 36 : Danube salmon from the Kupa River, about 10 cm total length (foto P. Mustafić).

Figure 37 : Large Danube salmon from the Hron River near Brezno (Sl about 300 mm)

2.16.3. Species identification

Adult Danube salmon specimens can be distinguished from the taimen (*Hucho taimen*) by missing reddish coloration on the abdominal part of the body. There are also genetic markers that can distinguish between Danube salmon and Taimen (Marić et al., 2015). Large mouth, exceeding beyond the posterior margin of the eye, large head and large body size, absence of red spots, white margins of fins, caudal fin deeply emarginate, no reddish coloration on belly are characteristics (Kottelat & Freyhof, 2007) that can distinguish Danube salmon from other potentially misidentificable salmonid species occurring in the Danube basin as brown trout - *Salmo trutta,* Linnaeus 1758,Black sea trout - *Salmo labrax,* Pallas, 1814*,* or non-native rainbow trout - *Oncorhynchus mikkys* (Walbaum, 1792)

2.16.4. Ecology

Danube salmon is exclusively freshwater species occurring in the well oxygenated, cold sub-mountain swift rivers and streams (grayling and barbel zone) with diverse river beds. Suitable habitat for Danube salmon has well oxygenated, fast-flowing water with mean summer temperature under 15 °C, but adults can tolerate a short time increase to 20 – 22 °C (Prawochenski & Kołder, 1968). Compared to other salmonid species, Danube salmon can withstand lower amount of dissolved oxygen down to 5 mg/l (Holčík et al., 1988), however, dissolved oxygen above 8 - 9 mg/l is one the major factor affecting the Danube salmon occurrence (Prawochenski & Kołder, 1968). Additionally, gravelly to gravel-sandy river bottom, rapid and long rifles alternating with pools and sufficient food supply. Holčík et al. (1988) describe the preferred habitats of adult Danube salmon as:

(1) deep holes below rapids, cascades, weirs, sluices and waterfalls, often shaded, by overhanging trees;

(2) calm water behind rocky projections from banks and groynes, projecting into swift rivers, streams and forming whirlpools;

(3) below and above lateral tributaries;

(4) in scours in and outside the stream line;

(5) behind boulders, piers, ice-aprons and other obstacles in the stream bed;
(6) at the confluence of lateral branches of a stream bed and above their bifurcation;
(7) on the convex side of stream bends; and
(8) in abruptly narrowed places of the stream bed.

During high water levels or of turbid water, the fish keep closer to banks, in the morning and in the evening they are usually found in stream bends, at the boundaries between rapids and pools. Later at night, they move to the shallow water. During the day and in the summer heat, they stay in deep holes below rapids or swim to the mouths of cool tributaries. In winter they seek for deeper habitats (Holčík et al., 1988). YOY Danube salmon occur close to the spawning site, later drift downstream and move to side arms, calm waters near banks, shallow habitats and small tributaries with high quantities of prey as other fish juveniles (Bastl & Kirka, 1959). Older, immature Danube salmon inhabit the same habitats as adults with some hierarchy maintained (Holčík et al., 1988). It has a lifespan exceeding 20 years. The Danube salmon is an apex predator and feeds on wide variety of food items. Young individuals feed on small macroinvertebrates and terrestrial insects and small fish, soon at 50 - 100 mm their diet is shifting to piscivory and it hunts as an ambush predator (mainly cyprinids), but their diet can include amphibians, small mammals, reptiles and birds (Holčík et al., 1988; Šubjak, 2003,Kottelat & Freyhof, 2007, Povž et al., 2015).

2.16.5. Reproductive biology

Danube salmon matures sexually at the age of 4 - 5 years and sizes around 60 cm. Males tend to mature sexually one year earlier compared to females (Holčík et al., 1988). Spawning takes places in very clean gravel in fast-flowing water in tributaries or in the main river in the water depth up to 120 cm, large individuals mostly select the main river for spawning. Spawning migrations are up to several tens of kilometers, however migrations >100 km were documented in the past (see Holčík et al. 1988 for details). Danube salmon are spawning each year and tend to migrate to the identical places, so Holčík et al. (1988) suggest homing behaviour. Spawning site is a combination of the suitable spawning substrate and hydrological conditions to ensure minimal water depth (Figure 38). Danube salmon is a lithophilous, brood-hiding species group A.2.1 (Balon 1975). Female excavate redd, with partial assistance of male, where the spawning takes place (Holčík et al., 1988), however, the contribution of males was rejected by Esteve et al. (2013). The spawning takes place in spring, usually from February to April when the water temperature reaches 6 - 10 °C. The duration of the spawning depends on the temperature, the more stable temperature the shorter the spawning. Spawning was reported during the day, but sometimes during warm nights. After the spawning, the pair spend about two weeks together, first days near the redd, later migrate downstream to the typical summer habitats (Holčík et al., 1988). Juveniles hatch after 25 to 40 days and larvae remain among gravel until absorption of yolk sack

Figure 38: Male guarding the redd Turiec River, Martin, 10.4.2020 (Sl> 1000 mm)

2.16.6. Threats and conservation status

Danube salmon is more or less common approximately at one third of its historical range (Holčík et al., 1988). It is listed as Endangered in the IUCN Red List (Freyhof & Kottelat, 2008) and in the EU Habitats Directive. The main treats are continuous habitat degradation and river continuity fragmentation. It is highly sensitive to anthropological impact, especially to low oxygen concentration and pollution, which

makes them good indicators of river ecological status. In some regions, poaching and angling can get populations under high pressure. Climate change shifts suitable habitats more upstream by increasing water temperature and decreasing the amount of dissolved oxygen, e.g. Slovak-Hungarian stretch of the Danube River. Healthy Danube salmon populations need considerable space and available prey. Genetic analysis from several countries identified bottleneck effects in all broodstock samples (Kucinski et al., 2015) and continuous stocking using this broodstock can negatively affect the genetic diversity of wild Danube salmon. Danube salmon needs free-flowing rivers with clean water and are very sensitive to hydropower exploitation (Holčík et al., 1988; Witkowski et al., 2013; Ratschan, 2014; Freyhof et al. 2015).
It is a Natura 2000 species requiring protection from EU states.
Appendix III of the Bern Convention (protected fauna)

2.16.7. Missing/unknown data

- Current distribution and origin of the Danube salmon in some Danube tributaries as new sites were confirmed from Ukraine and Romania in the Tisza Basin.
- The proportion of naturally reproduced individuals and stocked individuals.

2.16.8. References

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CHAPTER 3

Detection of potential key habitats and their spatial extension

Overview

This chapter focuses on the determination of key habitats, necessary for the successful lifecycle of migratory fish species. Firstly, we introduce 4 most significant habitat types used by migratory fish species and explain the collection and analysis of existing information, the types of information available and collection approaches. The next step is represented by field surveys to fill the gaps in knowledge and/or data. Different methods are described which enable the determination of riverbed morphology, substrate, hydraulic measurements, macroinvertebrates and plankton. These are all variables that help to gauge habitat suitability for migratory fish species. The methods differ with regards to the size of the water body, complexity of implementation and financial sources available, and should be selected according to the reader's own capacities and limitations. To successfully gather as much existing information on key migratory fish habitatsin a given area as possible, the approach considered should include both, historic and present data, which together form a backbone for data analysis and future research (Friedrich, 2012).

3.1 Habitat types

In ecology, a habitat isthetype of natural environment in which a particular species of organism lives. A species' habitat are those places where the species can find food, shelter, protection and mates for reproduction. It is characterized by both biotic and abiotic features (Ryan, 2019).

Migratory fish species require a number of different habitats to be present in a river to accommodate their different life periods and stages. Habitat requirements differ among migratory fish species as well as among fish life stages, however, some similarities can be identified either among species groups (e.g. litophilic and benthic fish species) or life stages (e.g. larvae or YOY). These different requirements are therefore reflected in habitat selection. The suitable habitats are mostly defined by water depth, flow velocity and discharge dynamics, substrate type, water temperature, available food resources, dissolved oxygen, available shelter and possible disturbance by human activities.

Typically, we recognize 5 well defined habitats utilized by migratory fish species at specific time of day, a period of year and/or in a particular life stage. These are:

a. As the name implies, **spawning habitats** are used for reproduction. Generally, fish species in the Danube Basin can be divided in rheophilic, phytophilic and pelagic spawners, whereby the first spawn in fast flowing, gravel bed areas with ample oxygen supply, the second prefer slow moving areas with vegetation and the last spawn in the open water. For the lithophilic species, the size of the substrate particles has to be large enough for the spawned eggs to sink between them (to the interstitial), thus gaining protection against predation. The fast flow typical for these locations also provides ample supply of dissolved oxygen to the eggs in the interstitium. Some rheophilic species even spawn in waters so shallow that the dorsal parts of their bodies emerge from the water.

b. Discharge dynamics play an especially important role for the phytophilic species which spawn among the submerged vegetation that can often only be reached during spring or summer floods.
c. **Nursery habitats** are used by early fish life stages for feeding and shelter. These habitats are usually located closer to the banks; they are shallower and characterized by lower water velocities. Water depth is crucial to maintain suitable water temperature for egg and larvae, with warmer waters generally increasing the rate of development. Abundance of zooplankton, which is the main food source of fish fry, is also more abundant in slow moving, deeper waters. Parapotamal side arms are only one example of a perfect nursery habitat with an additional benefit of fish juveniles being able to migrate between the main channel and the connected side arms.
d. **Feeding habitats** are used by adult fish for feeding and can differ widely among different species according to their dietary requirements and temporal dynamics of food availability. It means that feeding habitat can be shifted within the year, e.g. fish can feed on spawned eggs or emerging Ephemeroptera in the narrow window within the year.
e. **Wintering habitats** are used by all fish species to overcome the cold part of the year,which is generally the deepest, slowest moving parts of the river that enable energy saving. Deep parapotamal side arms can also serve as wintering habitats for some fish species.
f. **Resting habitats** are used by adult fish to conserve energy between key activities, usually in the form of micro-shelters behind rocks or boulders, in eddies, backflows or pools, or among vegetation and submerged trees. Parapotamal side arms can also serve as resting habitats for adults.

In general, crucial habitats that enable fish species to complete their whole life cycle should be interconnected and accessible, e.g. the side arms could be a suitable nursery or resting habitat, if the outlets are not blocked. Multiple, deep wintering holes available as far upstream as Vienna, became inaccessible after the construction of the Gabčíkovo dam. The lack and inaccessibility of parapotamal side arms along the Danube River strongly influence fish populations. River regulations that aim to create uniform riverbeds decrease the amount of micro areasfor resting or decrease food availability. Construction of dams increases water temperature in the uper strata of the waterbodyand thus decreases the amount of dissolved oxygen (Friedrich et.al., 2018).

3.2 Collection and analysis of the existing information

Identification of sources

Potential migratory fish habitat locations can be identified from a number of sources. Most data can usually be found in scientific institutions such as local universities, national and research libraries, specialized museums and research organizations, especially those involved in fisheries management. NGOs, commercial and private companies can also provide valuable information from their field, e.g. hydropower companies, transport and navigation and gravel extraction companies.
Moreover, national authorities such as ministries responsible for nature protection, agriculture, fisheries, navigation or infrastructure, national agencies and institutes as well as national archives are another potential source of information.

Figure 39 Photos of Sterlet (Acipenser ruthenus) provided by a fisherman - Dvor na Uni (photo credit: Rudolf Grgić).

Other, less reliable sources include professional and/or amateur associations, groups and individuals such as local fishing clubs, old fishing newsletters, fishing magazines, fishing equipment shops and individual anglers.

Type of information

Firstly, migratory fish habitat identification requires detailed information on hydro-morphological parameters of the target water body, some of the most important being water depth, flow speed, riverbed morphology, sediment type and sediment distribution, etc. Some of these parameters (most commonly depth of navigable rivers, etc.) can be found in the form of location data (GIS) and in cartographic materials such as satellite imagery or bathymetry maps. Digital orthophotos and other web based interactive maps (e.g. Google Earth) are widely produced by national authorities and other companies engaged in remote sensing and therefore, easily accessible in most European countries free of charge. A variety of maps is available for large, 1st and 2nd order navigable rivers such as the Danube, while this data gets generally scarcer for smaller rivers of 3rd order and up.

Secondly, existing data on migratory fish distribution, occurrence and abundance, can considerably impact the selection of locations for field surveys, as the presence of individuals themselves speaks to the suitability of the habitat.

Finally, the rapidly developing simulation models, drawing from existing remote sensing and observation data, can act as both, the source of information or an analysis tool. If models for sediment and/or habitat distribution exist for the target river stretch, they can give highly relevant predictions of locations about key habitats when linked and/or adapted with the specific requirements of that species (please see 4.3.2.1 page: 128 for a more detailed description of modelling).

Form of information

The most widespread form of informationare written sources in the form of scientific articles, books and field reports, but also fishermen report forms, fishing magazines or newsletters. The latter can be difficult to procure, but can sometimes provide unique information, later lost or even omitted from the scientific sources due to their relatively low reliability and/or resource intensive procurement. The literature can either be public or private, so sometimes permissions need to be soughtto allow access, usage, publication and/or redistribution.

The second, rapidly growing form of information, replacing and redistributing written sources of information, is digital form. Nowadays, scientific articles, field reports, fishermen magazines and/or newsletters are all published and disseminated digitally. Bathymetry maps for navigable rivers are also commonly available online. Moreover, topical websites such as forums, professional groups (LinkedIn, ResearchGate), networks of amateur observers (iNaturalist), or blogs can also offer a good starting point to identify potential personal observations that can later be confirmed through phone or meetings.

However, in addition to these traditional sources, digital platforms where all types of information from any number of above-mentioned sources on specific topics are collected and made available can provide exhaustive and easy to find information. The MEASURES project offers such a platform, where all existing information about migratory fish species is available. The MEASURES information system (MIS) already offers a wealth of information, counting more than 450 individual entries and will continue to add new information as it becomes available (accessible at http://85.204.145.162/measures/metadata/).

The third form of information is oralform, first-hand accounts of groups of individuals (e.g. anglers, researchers, local residents, etc.). This information can often be limited and unreliable due to the lack of experience and knowledge, or even intentionally incorrect. However, first-hand information (if accurate) can prove to be the most relevant and up to date source of data available, so it should be assessed and checked as much as possible.

Figure 40 Acipenser guldenstaedti preparation from Slovenian museum of natural history

Finally, preserved specimens (Figure 39) from research organizations, museums, fishing clubs and even individuals, will usually allow correct identification of species and they are usually accompanied by information on the exact location of capture. These records are the rarest and therefore, their collection is the most time consuming.

Collection methods

Once we identify and assess all potential sources and types of information, the most efficient approach is to start with the most abundant and easily procurable information, which is browsing and collecting data from online databases and publications. These usually also provide metadata about the source, so a snowball approach can be implemented, each source offering a number of new sources to be reviewed.

Second, written materials from libraries and similar should be reviewed, while simultaneously checking for personal observations through engagement with librarians and/or telephone calls. The later method is highly time-consuming compared to online or paper tracing, as tracking down specific people and pieces of information takes a lot of time and requires good communication skills and tactics. Eventually, field visits and meetings with individuals and representatives are required to collect and cross-check some of the data. Nevertheless, it can be well worth taking the time and gathering good quality first-hand information.

Overview

Table 1 Summary of information sources by type, form and collection method.

Group	Source type	Most common form	Collection method
Scientific institutions and research organizations	Relevant faculties (e.g. biology, ecology, fisheries, geography, hydrology, …)	Thesis, scientific publications	Online database, institutional library
	Institutes (e.g. biology, ecology, fisheries, geography, hydrology, engineering, …)	Scientific publications, field reports	Online database, institutional library
NGOs	Research organisations	Scientific publications, field reports	Online database, institutional library
	Societies, clubs and associations	Action plans, reports, observations	Online database, institutional library
Commercial, private companies	Hydropower companies	Project reports, cartographic materials	Online database, institutional library
	Transport and navigation (including tourism) companies	Navigation charts	Field visits, telephone calls, in person through meetings
	Gravel extraction companies	Navigation charts, work reports	Field visits, telephone calls, in person through meetings
National authorities	Public libraries	Books, journals, periodicals	Libraries
	National archives	Reports, cartographic material, books	
	Ministries and national agencies, libraries and archives	Public data portals, national plans and reports	
Non-scientific community	Fishing clubs	First-hand accounts, books	Field visits
	Fish shops	First-hand accounts, photos	Browsing
	Individual anglers	First-hand accounts, photos (Figure 2)	Through telephone calls, in person through meetings

Other sources	Fishing forums	First-hand accounts, photos	Browsing
	Fish research and conservation websites	First-hand accounts, scientific publications, field reports, Navionics - free WebApp	Browsing
	Projects (e.g. MEASURES project online database)	Scientific publications, photos	Browsing
	Networks of amateur observers	Occurrence data, photos	Browsing

3.3 Using cartographic materials to identify potential key habitats

Spawning, nursery, feeding and wintering habitats each exhibit a specific and distinct mixture of morphological and hydrological conditions (see Chapter 2 for detailed description of habitats). In addition, different migratory fish species and even their different life stages exhibit different habitat preferences. Some species, such as sturgeons, prefer greater depths; common nase (Chondrostoma nasus) requires fast-flowing water, while juveniles seek protection in the shallow and slow-moving sections (see Chapter 2 for detailed description of migratory fish species and their habitat requirements). The diversity of habitats in a river system and the number of parameters to be measured for each location is too extensive to perform field surveys of entire rivers. Especially in large rivers, field surveys are extremely resource intensive, but even for smaller streams, the number of locations for detailed field sampling needs to be narrowed down to ensure efficiency. Therefore, the preliminary identification of potential key-habitats using cartographic material is required to focus the subsequent field work to optimal habitats. Cartographic materials such as digital orthophotos, satellite images, and other web based interactive maps (e.g. Google Earth) and bathymetry maps (e.g. Navionics WebApp), are the most widely used methods to do this. Some of the most important features of potential habitats, such as deep pools, rapids, laminar flows, backwaters, gravel bars or sandy bars are easily identified through visual inspection of these sources and can reduce the number of locations for further analysis.

Aerial and satellite imagery

Digital orthophotos, satellite images and other web based interactive maps are the most widely available sources to gain the first impression of the targeted river or river stretch. The main morphological features of the river such as gravel and sand bars (Figure 41, Figure 42), deep meanders (Figure 43), backwaters (Figure 44) and riffles (Figure 46), with potentially suitable habitats can easily be identified from surface images. Such screening functions as the first filter to indicate the potential suitable habitat locations, for which more detailed information (depth, flow velocity, substrate) can be sought in the next step.

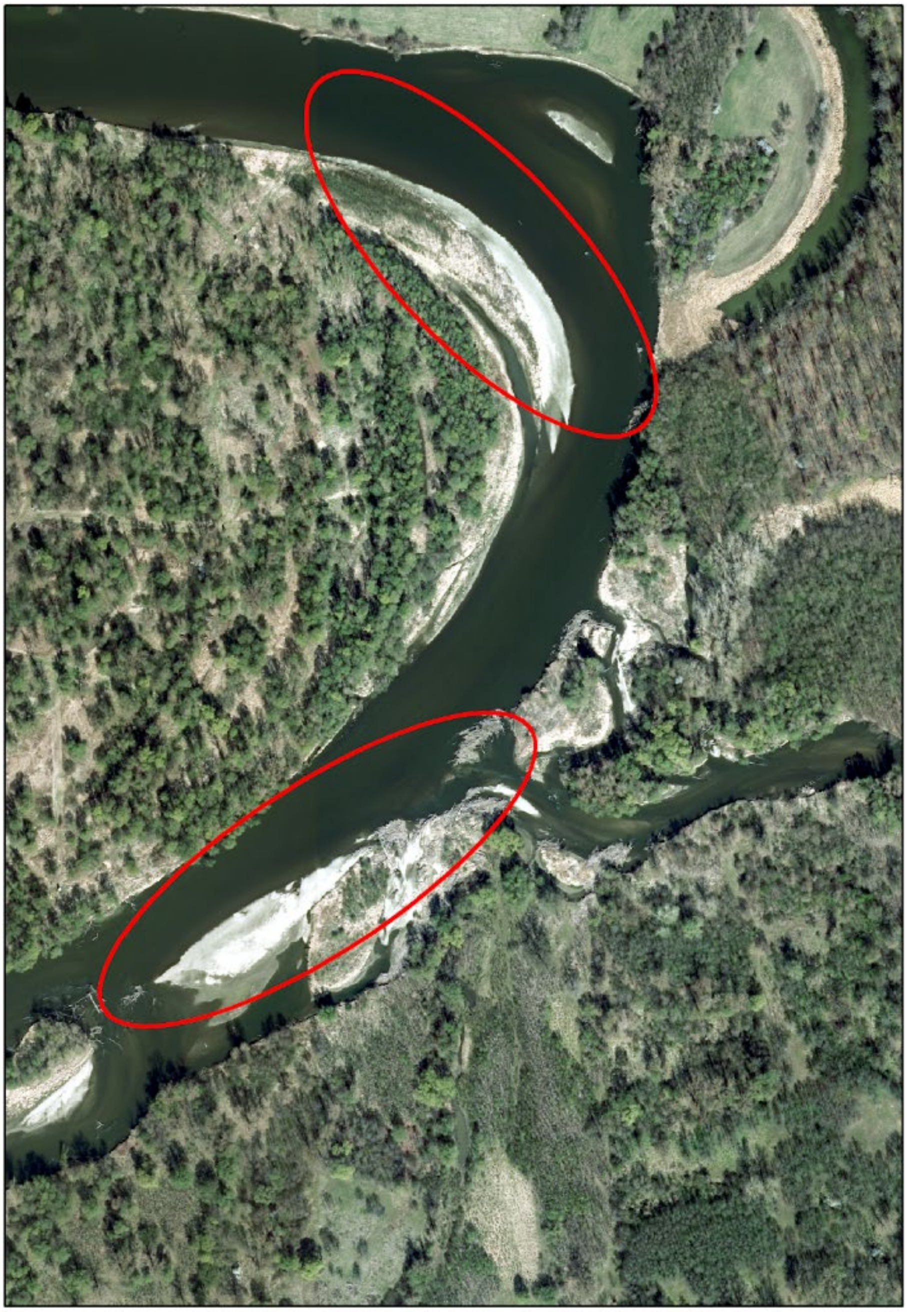


Figure 41 Gravel bar - potential feeding habitat (digital orthophoto) (photo credit: REVIVO, GURS, 2020).

Figure 42 Gravel and sandy bar - potential feeding habitat (Google Earth) (photo credit: Google Earth Pro, 2020).

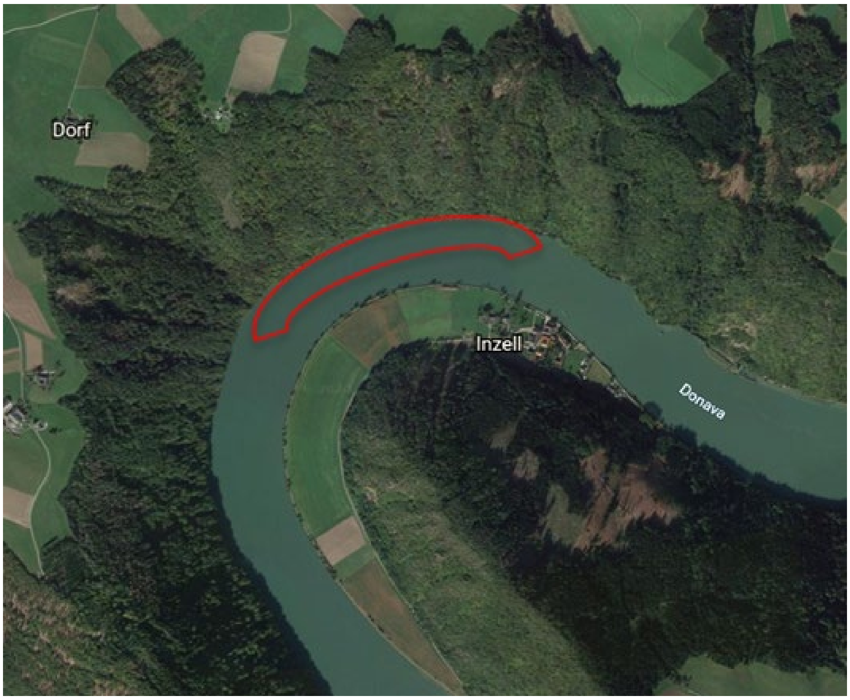


Figure 43 Deep outside part of meander - potential wintering habitat – (Google Earth) (photo credit: Google Earth Pro, 2020).

Figure 44 Backwater - potential nursery habitat (Google Earth) (photo credit: Google Earth Pro, 2020).

Figure 45 Gravel/sandy bars and backwaters - potential nursery and feeding habitat (digital orthophoto) (photo credit: REVIVO, GURS, 2020).

Figure 46 : Riffles - potential spawning habitat for common nase (Chondrostoma nasus) (digital orthophoto) (photo credit: REVIVO, GURS, 2020).

Bathymetry maps

Similarly as aerial and satellite imagery,visual inspection or automated computer screening of bathymetry maps (e.g. navigation charts) allows identification of the deepest parts of the river bed usually occurring as pools on the outside of meanders (Figure 47), or as individual depressions within longer stretches of a flat river bed (Figure 48). These habitats are typically used by all species as wintering habitats to conserve energy during winter (Weber et. al., 2009), but sturgeons also use them specifically to wait in the immediate vicinity of spawning habitats (Friedrich, 2012). Bathymetry maps can be obtained from national authorities, hydropower companies and other companies that need or produce the maps (Table 1) or can be found online (e.g. Danube FIS Portal). This step supports determination of potential habitats by confirming or providing more detailed information on depth for the locations, identified from aerial and satellite imagery.

Confirming suitable habitats by field measurements

Once locations of potential habitats are identified as described above, the next step is to conduct a field survey of these locations and confirm their suitability as key habitats for migratory fish species. This chapter thus aims to describe the tools and methods for on-site determination of the many aspects of key habitats,

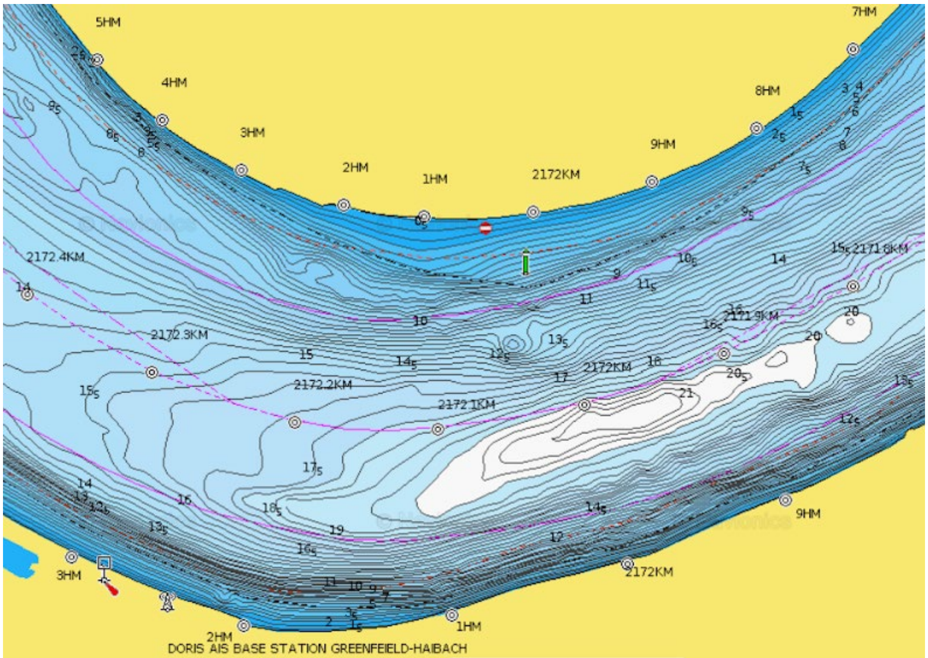


Figure 47 Deep outside part of meander - potential wintering habitat of migratory fish species(photo credit: Navionics WebApp, 2020).

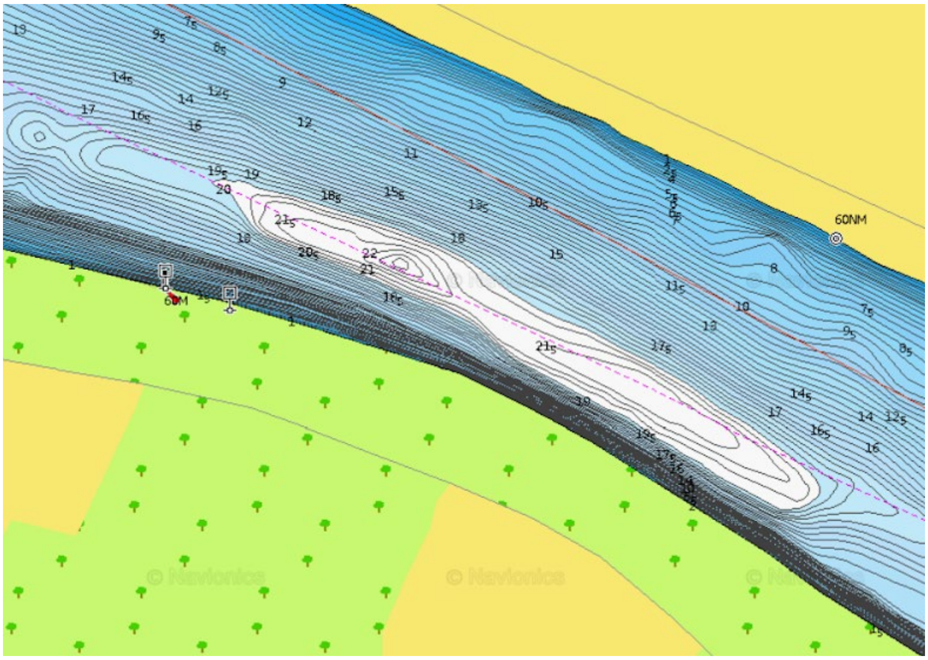


Figure 48 Sudden deepening in general levelled river bottom - potential sturgeons waiting location before spawning (photo credit: Navionics WebApp, 2020).

from mapping the riverbed morphology and substrate sampling to water flow and biota sampling, e.g. plankton and macroinvertebrates.

Mapping riverbed morphology

Even when bathymetry maps exist, they lack the precision required for sturgeon habitat mapping. Therefore, the next step to confirm suitability of identified potential habitats obtained through the previous steps, should be to screen morphological features of the riverbed by field measurements using different sonars, depending on the size and morphology of the target river (e.g. small tributary (2nd order or up) or the main Danube channel). The riverbed morphology data is used to further narrow down the suitable habitat locations for additional research such as macroinvertebrate and substrate analysis, and eventually fish sampling.

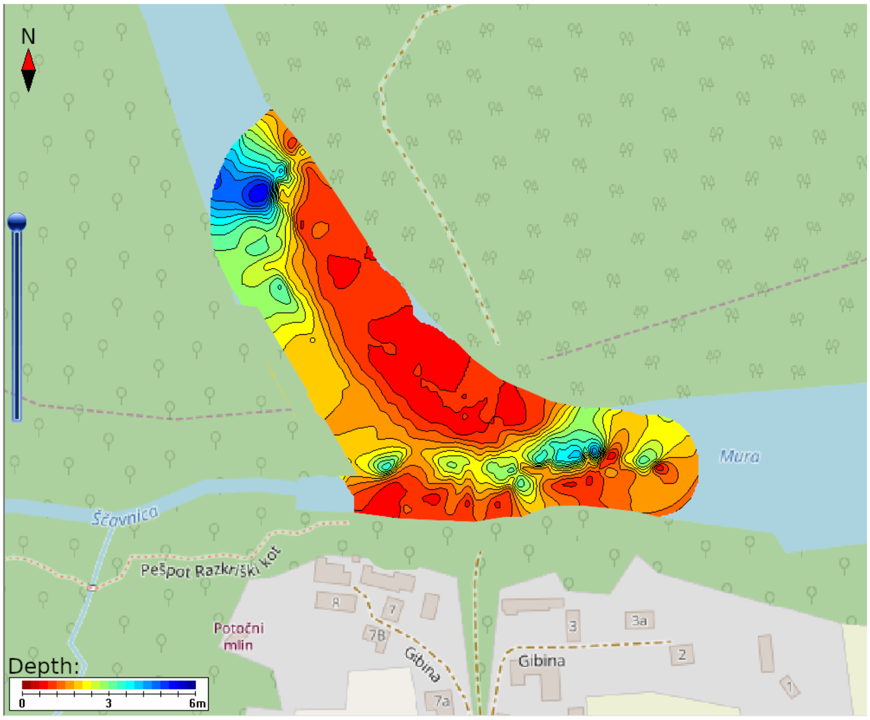


Figure 49 Bathymetric map of the Mura river at Gibina produced with sonar Humminbird Helix 7 CHIRP SI GPS G2N and AutoChart Pro software (photo credit: REVIVO, 2019).

An example of a suitable sonar, similar to the one used by Kaeser et. al. (2012) to map a relatively small, 4th order tributary with an average main channel width of 50 -100m, is a Humminbird Helix 7 CHIRP SI GPS G2N system with the XNT 9 SI 180 T

probe and AutoChart®Live option. This sonar supports 2 frequencies for 2D scanning (200 kHz/83 kHz with beam angle of 20°/60°). For our purposes 2D scanning with 200 kHz and beam angle of 20° was used as this frequency achieves higher precision than 83 kHz. At a depth of 2m, the 20 degree beam angle allows for a scanning transect of approximately 0.75m and at a depth of 5m, approximately 1.9m of the bottom. To increase precision, the transects should overlap, so multiple passes over the same location are recommended. During the scanning, the AutoChart®Live programme instantly records and writes the georeferenced depth data on a ZeroLine Europe SD memory card. The data is later transferred to a computer and processed via the Humminbird AutoChart Pro software (Figure 49), which also allows for creation of 3D bathymetric maps. Mapping riverbed morphology using a low-budget sonar can be time consuming and as such only feasible on comparatively narrow rivers, where the shallow depths and different obstacles (e.g. woody debris, large rocks, etc.) prevent the use of high-tech sonars with greater precision and/or reach.
For large rivers such as the Danube, the aim of the detailed mapping of riverbed morphology at key migratory fish habitats remains the same, but the method differs slightly. It requires a setup on a larger vessel, and specially qualified personnel to operate. The method starts with the setup of the Reference System network and the RTK connections between reference shore points and the multibeam. Then the Teledyne ODOM Hydrographic ES3 multibeam transducer with Valeport Mini-SVS sound velocity sensor is started and a system check is performed. After that corrections are applied for the height of the Javad Triumph 1 GPS antenna, the depth of the transducer and the magnetic declination. The river section with potential key habitats of interest can now be scanned with the Teledyne ODOM Hydrographic ES3 Control software by aligning the vessel according to the survey lines. The swath width is 120° x 3° for transmitting as well as receiving. The collected data is stored within a project and consists of raw files for each line surveyed. These files are processed within the Hypack software in the module MBMax. This module allows the filtering and editing of the point cloud

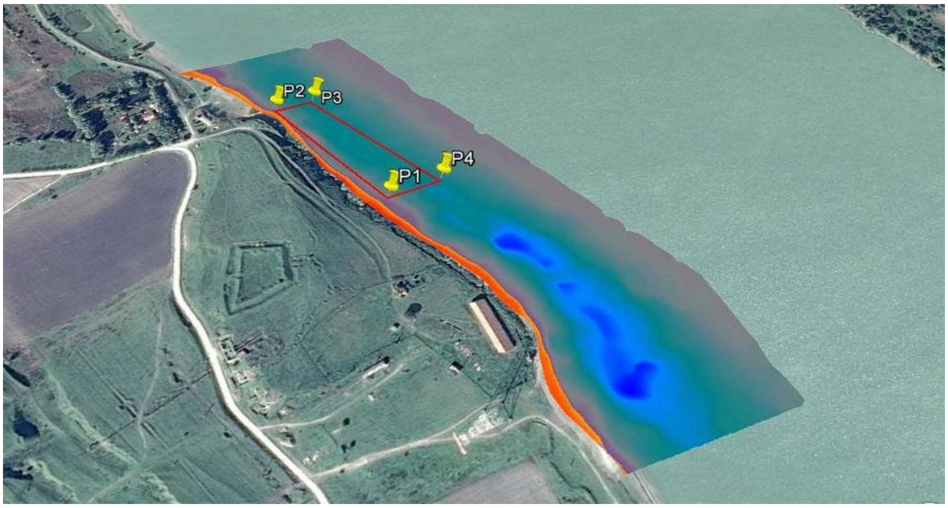


Figure 50 3D bathymetric map of the LDR at Tulcea, D Km 63 (Source: DDNI).

in order to exclude the spikes and corrupt data. This is a time demanding procedure. Afterwards, the final product is exported with Hypack, or the point cloud can be exported in XYZ files. It is then interpolated using ESRI`s GIS tools integrated with other data to produce 3D bathymetric maps of riverbed sections using ESRI`s GIS tools (Figure 50), which are then used to assess suitability of a given riverbed section as sturgeon habitat (Suciu R. et al., 2012; Suciu R. et al., 2015).

Modelling

Alternative approaches for mapping habitats at larger scales, making use of existing semi-quantitative survey data and GIS map-based covariate data can also be utilized to identify suitable sturgeon habitats, such as MesoCASiMiR (Eisner et al., 2005), MesoHABSIM (Parasiewicz, 2001), NMCM (Alfredsen et al., 2007) or RHM (Maddock et al., 2001), to name just a few. These methods apply different modelling techniques to extrapolate existing hydro-morphological data and predict suitable habitat locations based on predefined habitat requirements of target species. Sediment distribution, for example, is modelled based on flow and depth data. These techniques are especially suitable for mapping habitats in large rivers, where traditional field surveys are particularly resource intensive. However, they also require sufficient amounts of existing data.

3.4 Identifying the substrate

Identifying the substrate is a crucial step when locating the key migratory fish habitats such as spawning areas, which are chosen by most fish species mainly for their substrate. There are many ways to collect this information, with varying levels of complexity and cost.

Where water depth, flowand transparency allow, a visual census of the substrate may be the only method necessary. This involves assessing the substrate makeup of a small area by sight.At greater depths where the substrate is not visible from the surface and where flow speeds allow, an underwater camera, such as the WaterWolf UW 1.1. can be lowered horizontally from a boat and the substrate can be filmed and its composition identified. However, conditions are seldom favourable enough for such an approach, and other less direct methods need to be utilized.

Figure 51 Kynard probe (photo credit DDNI).

One of the simplest is the Kynard probe. It is a 80 cm 1" diameter galvanized steel pipeline, which is attached to a 25 – 45 m long 1 mm diameter galvanized steel wire that terminates in a rudimentary earphone (a peanut box is sufficient in this role) (Figure 51). By listening to the sound of the pipe hitting the river bottom, it is possible to determine the type of substrate at the study area (Kynard, 2002). Although unrivalled in its simplicity, the Kynard probe takes a certain amount of experience to implement successfully. It is also more suited to larger rivers with less bottom debris, such as the Danube, since it could tangle in smaller rivers with high amounts of woody debris.

An intermediate option with regard to price and ease of use is the Van Veen grab (Figure 52). It is very efficient in that it allows for simultaneous sampling of sediment and macroinvertebrates. It is, however, not suitable for hard or extremely soft top layers of sediment. The jaws should be opened and fixed in place via the catch, then lowered steadily into the water. Upon contact with the substrate the catch will release and the jaws will close, taking the sample. The jaws need to close fully, otherwise parts of the sediment escape and the sample should be discarded and sampling repeated (Van Veen grabs Manual, 2018). On the one hand, the Van Veen sampler provides high quality, quantitative data for substrate and macroinvertebrates simultaneously. On the other hand, it is time consuming and point specific, so many samples are required to determine substrate distribution.

Figure 52 Using Van Veen grab (photo credit: REVIVO, 2019).

Finally, the latest, the most efficient, but also the most expensive method is the side scanning sonar. The sonar provides accurate bathymetric maps showing the distribution of different types of river substrate on the river bottom (Ionescu, 2018). Research conducted by Kaeser used a Humminbird 981c SI system to acquire sonar imagery. The transducer is to be positioned at the bow of the boat and the system networked to a WAAS-enabled Garmin GPSMAP 76 to provide waypoints for image capture locations and track-point coordinates with depth measurements along the survey route every 3 seconds. Consecutive, overlapping sonar snapshots and coordinates are recorded to the system while maintaining constant position and speed. Raw sonar images are then transformed into sonar image maps with real-world coordinates using ESRI ArcGIS and IrfanView software. Substrate boundaries on these images are manually digitized around areas of uniform sonar signature by visual interpretation. These digitized lines are then converted to polygons and assigned a substrate class. The images are interpreted using texture, tone, shape, pattern and association to distinguish and classify the substrate polygons. The substrate classification includes six substrate classes which are defined on the basis of material composition and particle size: sandy (S), rocky fine (Rf), rocky boulder (Rb), lime rock fine (Lf), lime rock boulder (Lb) and mixed rocky (Mx) (Figure 53). Newer low budget sonars such as Humminbird Helix 7 CHIRP SI GPS G2N etc. produce even more accurate and sophisticated side scanning pictures.

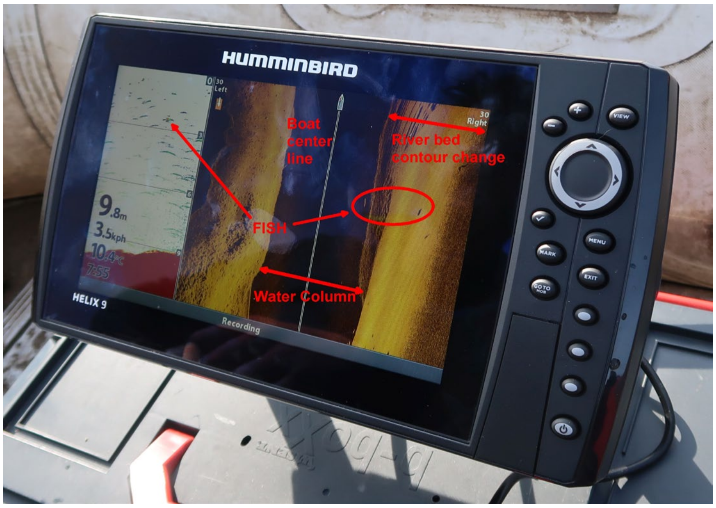


Figure 53 Raw sonar image annotated to identify key features (photo credit: Zrinka Mesić)

To assess the accuracy of these methods, a submersible drop camera can be used to image the river bottom and provide accurate visual data on a given area. If possible, the ground can also be disturbed and scraped with a long pole or shaft, providing additional auditory and tactile information.

Water temperature and hydraulic measurements

The upstream migration of sturgeons and other migratory fish species is strongly related to the development of river discharge and water temperature during the migration and spawning seasons. Hence, the hydraulic measurements are another key aspect of migratory fish habitat. Making use of all the previously collected data (bathymetry, sediment etc.) to identify potential sturgeon wintering pits and spawning sites, a focused hydraulic survey can be designed.

1. Water temperature

Water temperature is a fundamental physical characteristic describing properties of surface waters, having adirect impact on the flora and fauna of aquatic systems. To measure the water temperature from surface to bottom, the most apropriate device is YSI Multiparameter Digital Water Quality Meter (Figure 54).

The YSI Professional Plus handheld multiparameter meter provides extreme flexibility for the measurement of a variety of combinations for dissolved oxygen, conductivity, specific conductance, salinity, resistivity, total dissolved solids (TDS), pH, ORP, pH/ORP combination, ammonium (ammonia), nitrate, chloride and temperature.

Figure 54 The YSI Professional Plus handheld multiparameter meter (Source: https://us.vwr.com/store/product/14796274/professional-plus-multiparameter-handheld-instrument-ysi)

The ProDSS Portable Digital Sampling System is designed for use in demanding field applications such as surface water, groundwater, and aquaculture. The rugged and reliable ProDSS allows for measurement of up to 17 parameters. The ProDSS features user-replaceable digital smart sensors that are automatically recognized by the instrument when connected. A backlit color display, large memory, convenient calibration procedures, rechargeable battery, and powerful PC data management program (KorDSS) make the ProDSS user friendly. The optional GPS function, wide range of sensors, and varying cable lengths allow for complete customization of the ProDSS. Mil-spec (military spec) connectors and a waterproof (IP-67), rubber over-molded case ensures durability to provide years of sampling even in the harshest field conditions.
(https://us.vwr.com/store/product/14796274/professional-plus-multiparameter-handheld-instrument-ysi)

2. Simple flow meter

For measuring the water velocities at a fixed point close to the bottom of the river a flow-meter (FLO_MATE Model 2000) was used. The FLO_MATE Model 2000 uses an electromagnetic method to measure the water velocities, has a measurement range of -0.15 to 6 m/s and an accuracy of ±2 % (Du et al. 2014). This flow-meter has the advantage that it can measure the water velocities close to the river bottom (0 – 50 cm above the bottom), measurement that cannot be done with other types of equipment (May , 2017). The measurement is performed from a boat that is anchored above the place where the measurement is planned to be done (wintering habitat, feeding habitat or spawning habitat). The sensor of the flow-meter is attached to a special weight fixed on a 35 m long cable that is released in the water using a winch with a counter that can measure the length of the cable released. To measure velocity close to the bottom the cable is released until the weight with the sensor is on the bottom of the river. When weight with the sensor is on the bottom of the river the counter is reset to zero and then the weight with the sensor is raised to the desired distance from the bottom of the river (e.g. 20 cm, 30 cm or more) to measure the water velocities (Figure 55, Figure 56, Figure 57).

Figure 55 Portable Flow-meter FLO_MATE Model 2000 used for measurement of water velocities close to the bottom of the river (photo credit: DDNI).

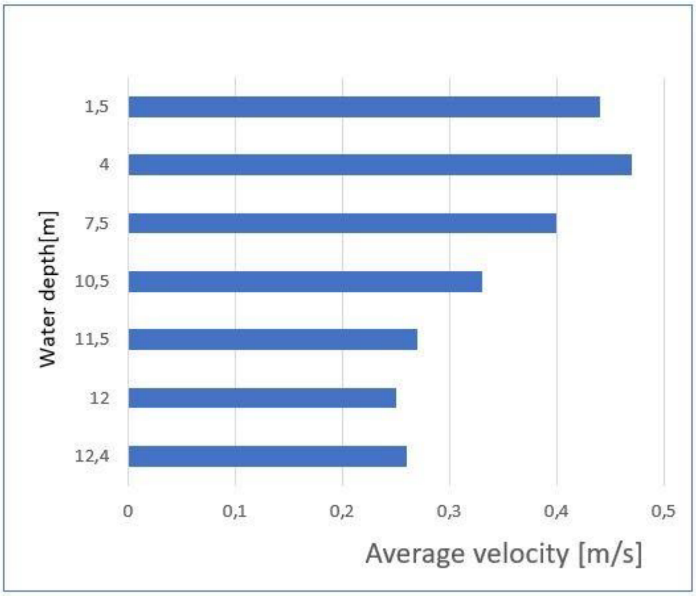
Figure 56 Example of suitable boats for performing hydraulic measurements (photo credit: Radu Suciu, Stefan Honț/ DDNI Tulcea).

Figure 57 EFM setup to operate from the back of aluminium boat SAM (photo credit: DDNI).

To conduct water velocity measurements, the boat must be on anchor at the desired site. For the anchors to hold, the length of anchor ropes must be equal or longer than 3 times the water depth of the river at that location.

The measurement requires participation of two operators. The weight (2) with the sensor (4) attached pointing upstream (u/s) is first lowered by the first operator with the winch (1) to the bottom of the river. The second operator determines the water depth using a hand held or fish finder sonar and turns the depth adjusting wheel of the display to show the value of the maximum depth. Then the first operator switches the EFM on. The unit starts a series of 30 successive velocity measurements and displays the average value of the water velocity about 15 cm above the bottom. After this process is completed, the first operator uses the winch to lift the weight and sensor to the next desired depth and the unit continues measurements. The data should be recorded in a table on waterproof paper.

An example of velocity measurement conducted on Sept/20/2018 during the training workshop on the Danube is shown in Figure 58 and Figure 59



Position of the boat:
N: 45° 16' 22.3
E: 28° 29' 26.1

Depth [m]	Average velocity [m/s]
12.4	0.26
12	0.25
11.5	0.27
10.5	0.33
7.5	0.4
4	0.47
1.5	0.44

Figure 58 Water velocity measured by DDNI SRG in the wintering hole of Uzlina, St. George arm km 67, was 0.13 m/s (photo credit, DDNI).

Figure 59 The potential wintering pit /hole located close to the right river bank downstream the Bechet harbour / CP 5 (photo credit: DDNI).

3. Acoustic Doppler Current Profiler (ADCP)

To evaluate the average velocity of the flow of the river, as well as the discharge at selected sections of interest one may also use the ADCP. One example is the River Surveyor M9 which can be operated from any type of research boat, because the sonar is placed on a separate float (Figure 60).

Figure 60 Left: Specifications and operation setup of River Surveyor M9 while recording current profiles at Red Rock spawning site for sturgeons in the LDR at km 311(Source: DDNI); Right: Sontek M9 with 9 beams (photo credit: https://www.sontek.com/riversurveyor- -s5-m9

The use of acoustic Doppler current profilers (ADCP) for measuring streamflow (Oberg, 2007) and discharge is becoming increasingly widespread (Yorke, 2002). The River Surveyor M9 ADCP is a hydro acoustic current meter similar to a sonar that is used while moving perpendicularly across the river to measure water current velocities over a depth range, utilizing the Doppler effect of sound waves scattered back from particles within the water column (Simpson, 2002). Briefly, ADCP measurements derive flow velocity from the Doppler shift of the measured return of an emitted acoustic signal that is reflected off suspended matter in the water column (Muste et al., 2004). The information from a combination of several (usually three or four) acoustic beams is used to calculate the mean velocity and flow direction, based on the assumption that the acoustic scatterers move at the same speed as the water and that flow is homogeneous over the area covered by the beams (Gunawan et al., 2011). These locally-averaged flow measurements are split vertically into cells (bins) that each represent a flow vector at a certain depth. When deployed from a moving vessel, a series of these velocity components measured perpendicular to the cross-section produces a transect of flow vectors across a stream, usually represented in the form of a raster, where each raster cell represents one flow vector (Shields et al., 2003). Generally, this series of measurements in a transect is used to calculate discharge. It isexcellent at measuring and recording velocity profiles and river discharge, but is unable to measure velocities in the 10 % of the water column directly above the bottom, which are often the most crucial velocities that determine the suitability of habitats.The spatial distribution of flow patterns is useful data in studying riverine habitats and geomorphology (Flener et al., 2015).Examples of velocity profiles recorded with ADCP River Surveyor M9 are shown below (Figure 61, Figure 62).

Since river discharge measurements using ADCP are costly to conduct on a regular basis, daily water levels and temperatures as recorded by the relevant

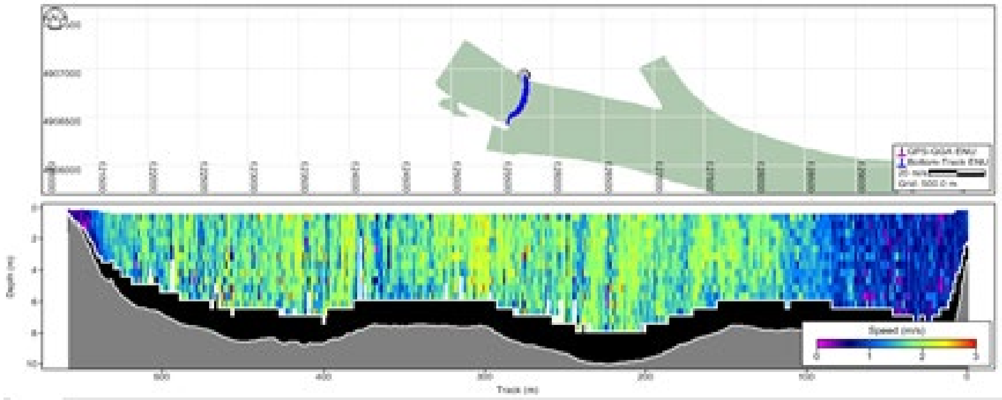


Figure 61 Velocity profile at 400 m d/s the main dam at Iron Gate 2 / LDR Km 863 (photo credit: DDNI, 2014).

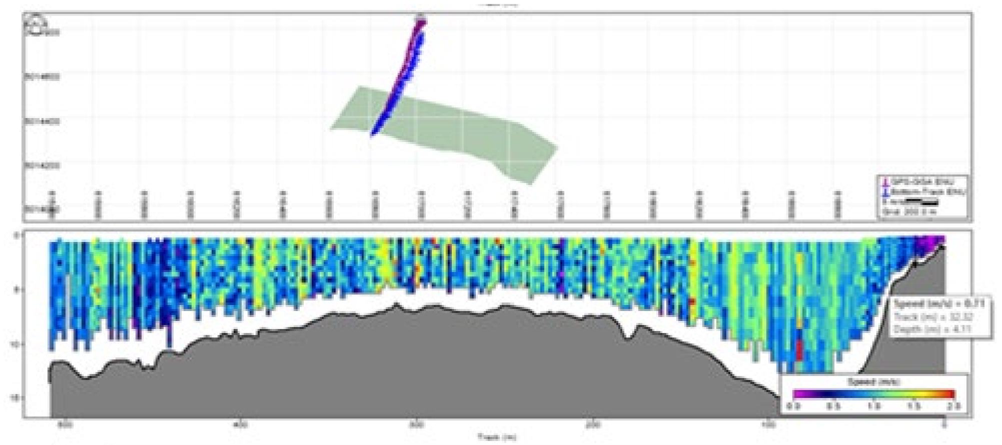


Figure 62 Velocity profile at the spawning ground of Noviodunum, LDR km 100.5 (March 2017)(photo credit: Suciu et al., 2015).

national organisations can be used to extrapolate river discharges instead.

Biota sampling

Availability of food is an important parameter determining suitability ofpotential feedingand nursery habitats for sturgeons as well as for other migratory fish species. Two main groups of biota should be sampled, zooplankton and benthic macroinvertebrates. These studies can be conducted separately or simultaneously, during the measurements of abiotic parameters. A number of standard operating protocols for sampling and analysis of zooplankton exist and should be referenced for detailed information. However, the goal of such sampling is to confirm the presence of preferred feed species and their relative abundance, so qualitative sampling is generally sufficient.

Zooplankton sampling

The zooplankton sampling designs differ with respect to water depth and presence of macrophytes. In shallow habitats with no stratification, one sample is representative for the entire water column, while one sample per stratum should be collected for deeper, stratified habitats. Additionally, for habitats containing either sediment or macrophytes, sampling should include these micro-habitats, as "large-bodied" mesozooplankton (typically cladocerans) often aggregate within submerged plant stands or sediment during daytime.

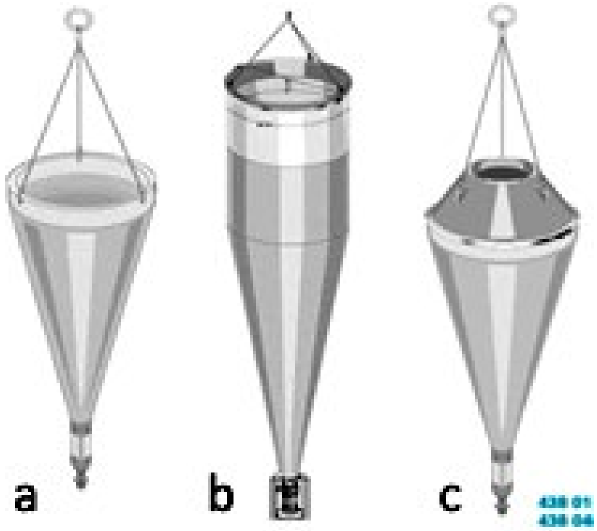


Figure 63 : Examples of different nets that can that can be used for water and (zoo)plankton sampling in deep waters: a) conical plankton net. b) cylindrical plankton net (Indian Ocean Standard Net), and c) a net with Apstein cone (photo credit: AQUACOSM, 2019

For a representative sample of the entire water column, a net without a closing mechanism such as conical plankton net or cylindrical plankton net must be used (see Figure 3.24). During the sampling, the end of the flanged net should be attached to a rope with markings for certain depths, to measure the sampling depth. The net should be retrieved by pulling it back to the surface with a steady constant speed, ca 0.5 m/s or slower if clogging is severe for smaller meshed nets. The content of the plankton net is emptied into a bucket with the help of water sprayed against the outside of the net(AQUACOSM, 2019). During the joint Danube survey, 50 litres of water were filtered through a plankton net with 50 μm mesh size at three (left, middle, right) profiles of the river at each sampling site (Zsuga, 2014).

The samples should be stored in a 70 % ethanol solution, or, in case PCR analysis is planned, in a refrigerator/freezer or 90 % ethanol solution. All microorganisms such as fish larvae, coelenterates, decapods and similar should be removed and counted before subsampling during laboratory analysis. The subsampling is carried out with pipettes or a plunger sampler. Then the sample is prepared for counting with sedimentation, centrifugation or filtration. The final step is the counting procedure (enumeration) using a Sedgewick-Rafter cell or a sedimentation chamber (1-25 ml) (AQUACOSM, 2019).

Benthicmacroinvertebrate sampling

The Benthic macroinvertebrate community is often the main food source for migratory fish species and as such should be analysed to determine whether it is aligned with fish dietary requirements. The most suitable sampling method should be selected based on the aim of the study and the sediment type. For migratory fish habitat mapping, a qualitative or semi-quantitative method is sufficient, as it allows for detection of the majority of species present and estimation of their relative abundances.

Figure 64 Hand net (left) and Surber sampler (right) with the frame size 15 x 15 cm (photo credit: www.hydrobios.de, 2020).

An inexpensive semi-qualitative method for sampling benthic macroinvertebrates is a combination of kick sampling, in which open surber and hand net (Figure 64) are used, and grab sampling using the Van Veen (VV) grab (Figure 63). This combination of tools allows for a relatively fast and simple assessment of macroinvertebrate communities at different depths, substrates and flows, which results in a more representative sample and precise data for the targeted potential feeding habitats. This method represents a modification ofmulti habitat sampling (MHS) employed by the joint Danube survey (Graf et.al., 2014), which is a much more time consuming (larger no. of sampling sites) alternative to the method described below.

Most of the migratory fish species, especially sturgeons, prefer to feed in deeper parts of the river, so it is important to include these areas in the sampling. A stratified random sampling design should be developed, depending on the location and depth distribution of the target location, but should in general include 3 strata: <20cm, 20-60cm and >60cm. At each potential habitat location, 2 random samples should be taken at each of the three depth strata, accumulating to 6 samples per site (e.g. 2

samples at 20 cm, 2 samples at 60 cm, and 2 samples at >60cm). The kick sampling method using open surber or hand net (Figure 65) is most suitable for depths below 80cm and the Van Veen grab is used in great depths. We recommend using the hand net with a 2m long handle. The size of the metal opening and the mesh size should be standard so as to allow for quantification. The opening should be placed firmly on the substrate, perpendicular to the flow, with the net opening against the river flow. Then, start kicking the substrate intensively a few centimetres from the net opening for 20-40 s, followed by a few seconds pause, during which the dislodged animals wash into the net and the water clears. Repeat the process one more time before concluding with a careful lift of the net. Where water flow is slow, the net should be lifted by drawing a figure 8 pattern in the water, so as not to lose the animals. As much abiotic material (leafs, wood, pebbles ...) as possible should be removed from the sample before conservation in a 75-96 % ethanol for further analysis in the laboratory.

Figure 65 Kick sampling method used on Mura River in Slovenia (photo credit: REVIVO, 2019).

In greater depths where application of the kick sampling method is not safe, accurate or effective, sampling should be done from the boat, using the VV grab sampler. The VV sampler should be weighted depending on the river flow and depth of the target location. The VV grab should be allowed to swiftly sink to the bottom on its own, do not try to lower it slowly. Once on the bottom, pull firmly to activate the closing mechanism and then raise it to the surface. The content should be poured into a sampling tray or a strainer and stones, leafs and woody particles washed and removed from the sample. Only clean samples should be preserved for laboratory analysis. Mark the samples as described above.
Both these methods are cheap and easy to apply as well as time efficient. The results from kick sampling provide a full coverage of species as well as quantitative data, useful for comparison or other research purposes. On the other hand, the success of sampling with Van Veen grab highly depends on the flow speed and substrate size, so

suitable weighting is crucial. In addition, it is not suitable for larger grained sediments where rocks prevent the closing of the grab.

However, in areas of slow current and fine sediments it provides good semi-quantitative data on the macroinvertebrate community that allows for assessment of its suitability for migratory fish species.

Another option for greater depth sampling that is used in conjunction with the MHS by the joint Danube survey is the deep water sampling(DWS) method. With this method, a triangle shaped dredge with an iron forked mouth and collecting net with 500 µm mesh size is pulled with a rope in the downstream direction. The dredging speed of the sampler should not exceed the river current velocity in order to avoid washing the material out of the net (Graf et.al., 2014).

Compiling a GIS map of sturgeonhabitats

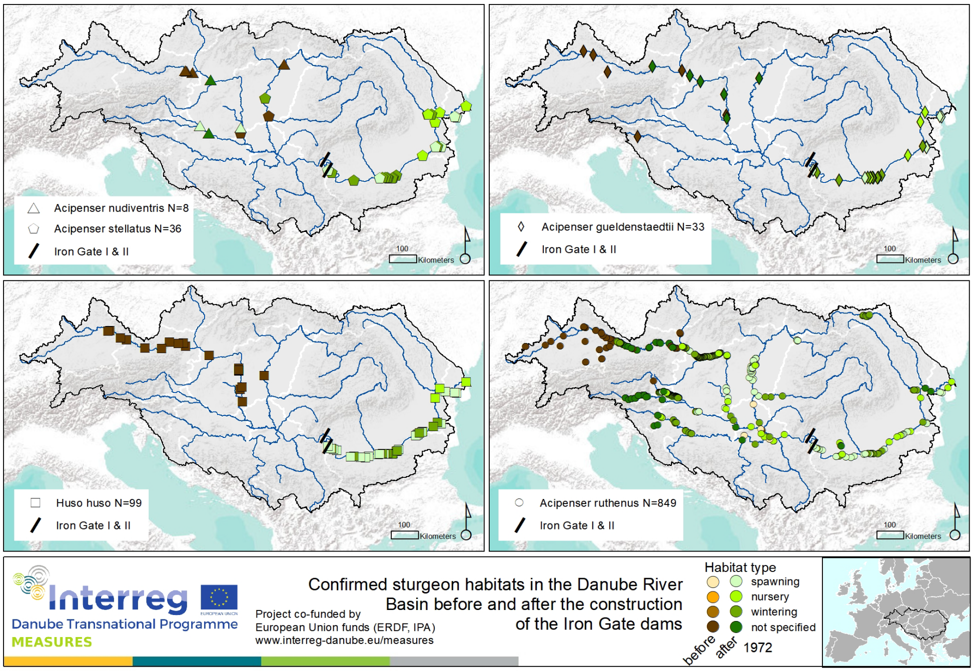


Figure 66 GIS map of potential habitats (photo credit: BOKU, 2020).

Locations of potential habitats can be presented in different ways. Probably the most appropriate method is using GIS maps. In this manner the user gets the quantitative and spatial overview of the results. The maps can be produced with several open source softwares such as QGIS, MapWindow GIS and Grass or subscription softwares such as ESRI ArcGIS etc. In Figure 66 you can see an example of how to properly map and present the quantitative and spatial data with ESRI ArcGIS.

The above mentioned GIS maps were developed including data about the geographical distribution of confirmed sturgeon habitats within the DRB. The data originate from before and after errection of the Iron Gate dams in 1972 and were collected from publications, project reports, books, field surveys, historical data, grey literature, or fisheries data from involved project partners. The habitat types were seperated into „spawning, nursery, wintering and not specified habitats" and were further described by using the following criteria:

- Location (identified via latitude and longitude coordinates)
- Water depth [m]
- Name of sturgeon species
- Year of habitat registration (Elguea, 2020).

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CHAPTER 4.

Confirmation of habitats by presence of species

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Overview

This chapter focuses on confirmation of habitats using different methods and tools to confirm the presence of fish species. All 4 types of habitats (wintering, spawning, nursery and feeding) require a specific approach to catch, record or observe the fish at various life stages. The best way to describe a habitat is by visual observation of the fish, but visibility depends on water level or water turbidity. The confirmation of the wintering habitats was done by catching fish in the winter, as well as filming or recording the fish during the winter period in the Danube River and its tributaries. For spawning habitats, different life stages of fish were caught and the results were supplemented by eDNA and visual observation. The nursery habitats are very important for early life stages and wereconfirmed by catching "young of the year" (YOY) in the Lower Danube River. The feeding habitats from Danube River and Black Sea coast were confirmedby comparing the food availability of the habitats with the stomach content of the captured fish.
In the last subchapter, the tagging methods were described, since the tagged fish could provide long term reliable information about their movement and behavior. Various tagging methods were developed in order to be used on different fish life stages and in different water bodies.

4.1. Wintering habitats

The winter period is a period of the year that has a great impact on limnological (ecological and biogeochemical) processes, which is reflected on the freshwater fish communities. To increase the chance of survival in the winter, which is the critical part of the year, and to avoid unfavourable conditions, the fish of the temperate regions inhabit parts of the river (bed) where they can rest and survive winter with minimal use of energy. These places (parts of the riverbed) are known as wintering habitats.
The efficiency of research and sampling of freshwater fish largely depends on the methods, gears, habitat type, target species (fish community) as well as their size and belonging to certain ecological guilds. In addition, there are methods and equipment (gear) that are more or less selective and specialized for certain groups or species of fish as well as those that capture a larger number of species and sizes of fish and are therefore considered non-selective.

Like most aquatic organisms, fish are ectothermic (poikilothermic) organisms whose body temperature depends on the environmental temperature, and on their behaviour. The energy required for survival is directly associated with temperature. Water temperature, however, can occasionally vary widely, being an environmental variable that influences growth and reproduction, or all physiological processes, metabolism and behaviour. Fish have developed multiple survival strategies to maintain their body temperature within the optimal physiological range, by selecting the appropriate habitats and ultimately conserving energy (Ultsch, 1989; Wootton, 1998; Haesemeyer, 2020).

In temperate zones, winter is one of challenging and critical periods for the survival of fish, and it can be considered a life stage which is strongly defined by the influence of the environment. During winter, many migratory and mobile species use only very limited habitats and restrict their movements, representing a "temporal bottleneck". During this critical period, thermal stress, starvation, hypoxia and predator pressures are the greatest threats and challenges. In order to survive these risks during overwintering and to improve their chances of survival, many species apply grouping strategies to concentrate their numbers, and select and use appropriate winter habitats or refugia. River species very often select larger water bodies and deeper habitats for overwintering, such as holes and depressions, where the physicochemical and hydrological conditions are most suitable. Water temperature is constant and somewhat higher than surface water, while the flow rate is usually slow, thus reducing energy demand (Cunjak, 1996; Shuter et al., 2012; Weber et al., 2013; Thayer et al., 2017).

Human impacts on freshwater habitats are pronounced and evident in regulation and channelization works, construction of barriers, and inflow of pollution. This results in marked hydrological and hydromorphological changes and ultimately in habitat degradation. The anthropogenic impacts lead to loss, degradation or reduced accessibility of winter habitats that are suitable for survival. Since these are habitats used by fish at times when they are under additional environmental pressures, survival becomes even more risky and critical. Unfortunately, for many species, there are little to no data on the movements and behaviour during wintering and knowledge of their winter habitats. Accordingly, research of wintering habitats and ensuring their protection are of exceptional importance in managing populations of migratory species and in conservation planning (Weber et al. 2013, Thayer et al. 2017).

4.2.1. Recording images /videos of sturgeons in wintering habitats

One of the least invasive methods to verify potential sturgeon and other migratory fish in wintering habitats is the recording of specimens while overwintering. This can be accomplished with different methods, requiring a diverse type of equipment and expert knowledge. Applied methods are qualitative, designed to provide information on presence of the wintering fish, not the abundance. Main applied methods are recording of video on pole/rope and recording by divers.

Recording organisms at wintering habitats with minimal equipment and no professional training is accomplished by securing a WaterWolf UW 1.1. underwater camera to a handle/pole/rope, and then simply holding it over the side of a boat, 10 - 20 centimetres above the riverbed. In the position of the potential wintering habitat the boat should be navigated to pass at its slowest possible speed over the wintering habitat position. Afterwards the whole recording is reviewed for potential fish observation (sightings). Another advantage of this method is that it enables an accurate assessment of substrate composition at the location of recording (Figure 67). The method is very easy to implement, however results can vary according to the water turbidity and operator experiences. The disadvantage of this method is that there is no live streaming, therefore recorded material can only be checked afterwards on the computer (when it is downloaded from camera to computer). The camera on the handle / pole can be used for water depths down to 3 metres. For greater depths a rope can be used, however in this case water velocity must be low and riverbed without obstacles such as trees, branches etc. In addition, the method can be used on other habitats as well.

Figure 67 Images captured with the WaterWolf UW 1.1. camera on the Mura river in Slovenia (photo credit: REVIVO, 2020).

The best approach to selectively search for and record wintering migratory fish is to hire a certified river diver with professional filming equipment and experience (Figure 68). Dive sites should be chosen according to depth (wintering fish usually aggregate in deep holes and depressions in the riverbed) and flow (too much can inhibit the diver's ability to swim and film). The dives should be planned at periods of maximum visibility to increase chances of fish sighting. The advantage of this (diver) method compared to the method of camera on handle / pole is that the diver can see what the camera is recording in real time and can actively search for fishes that are hiding under debris (Figure 69). It does, however, require more funds and takes longer to cover a given area. Besides wintering habitats this method can be used at other habitats as well.

Figure 68 Diver with equipment preparing for a dive in the Mura river in Slovenia

Figure 69 Crucian carp (Carassius Carassius) filmed during wintering in the Mura river in Slovenia (photo credit: REVIVO, 2020).

4.2.2 Fishing with nets at wintering habitats

Most research on rivers, inhabited by cyprinid species, is carried out in thewarmer part of the year, from April to October, when the water temperature is higher than 15°C and when most species are active. There are a reduced number of researches on fish in river habitats during the winter and they are very often oriented towards specific, characteristic, targeted species. Given the above, sampling during the winter requires methods that are adapted to environmental conditions, the ecology of each species and the hydrological and hydromorphological characteristics of the river.

It is good practice to start research of the wintering habitats with the desktop research of riverbed data. The bathymetry data are analysed in the GIS and exceptionally deep parts of the river should be identified (Figure 70). These parts of the river should be surveyed (at any time of the year) to confirm potential for the wintering habitat. Moreover, the potential habitat could be additionally scanned with the sonar. In this way the recent structure of the riverbed morphology- is obtained (Note: the bathymetry data of the riverbed could be several (or 10) years old and in that period the changes in riverbed could happen). In addition, the survey with sonar could help define the position of possible wintering habitats better.

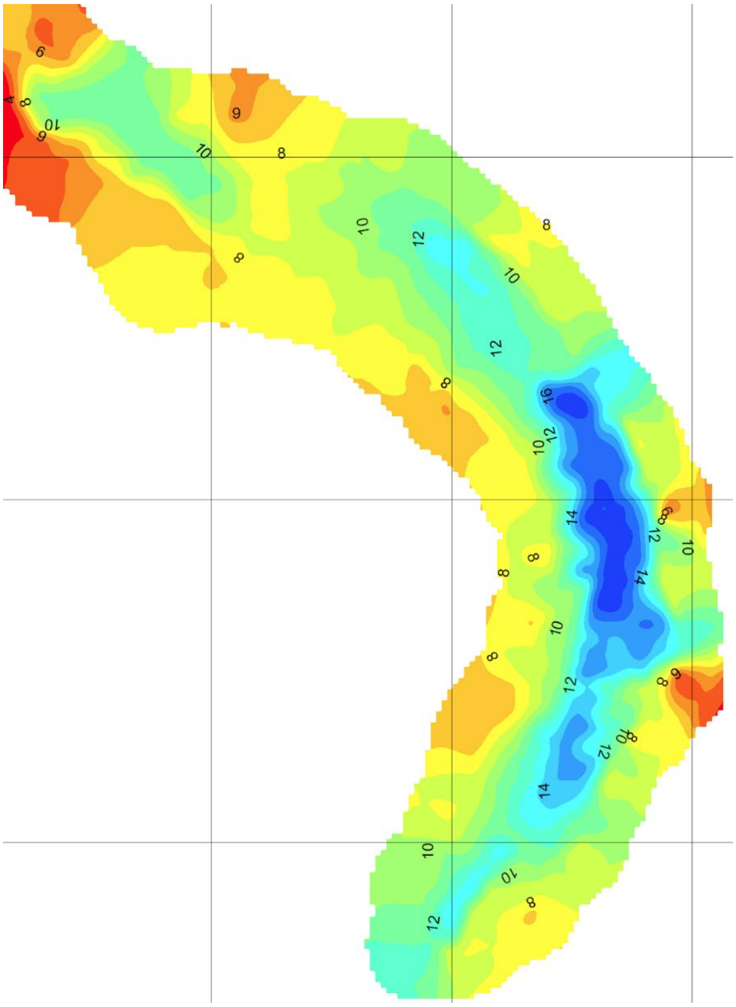

Figure 70 Bathymetry of potential wintering habitat

To confirm the potential wintering habitats of migratory species the survey (catching) of fish should be conducted between 15th January and the 15th March. The timeframe for the survey will depend on the yearly conditions – if the winter is exceptionally warm the dates should be adapted to the cold part of the winter period. The selection of the exact sampling sites is based on previously collected information (echo sounder), as well as an earlier GIS analysis of the type and structure of the river bed (hydromorphology). Sampling is performed on previously recorded deeper places on watercourses, which, with their position, size and structure of the bottom, best correspond to potential habitats (wintering grounds) for sturgeon, but also for other migratory species.

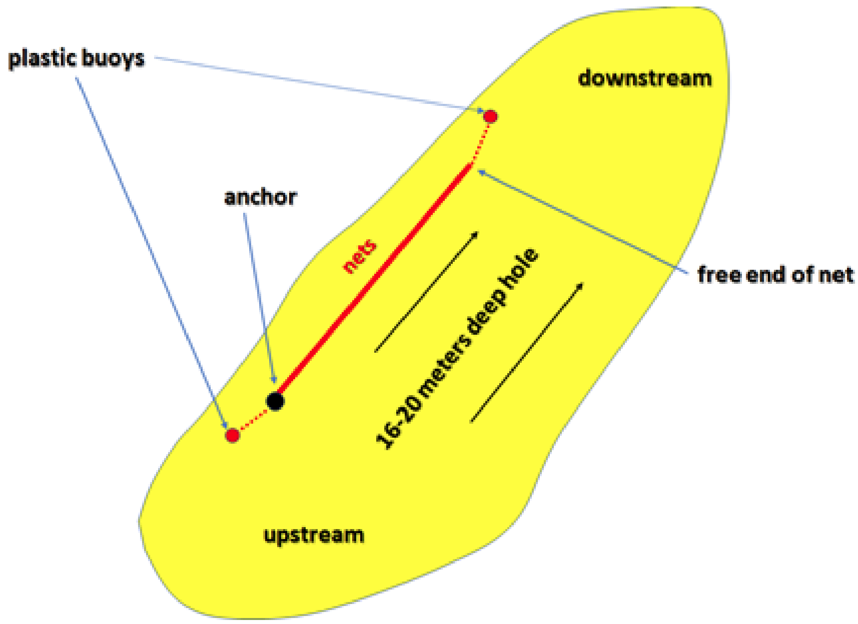

Figure 71 Schematic representation of the nets setup

Three-layer gill nets (trammel nets) were used to research winter habitats (Figure 71). Although the nets are mainly intended for sampling lentic habitats, they were used for wintering surveys due to the inaccessibility and depth of the benthic habitats, but also due to the fact that sturgeon and other migratory species gather and concentrate in these places. On the part of the river which is 150 meter wide, the trammel nets which are altogether 90 meter long (3 x 30 meter) and 2 meter high with 40 -60 mm inner layer mesh size and 200 mm outer layer mesh size were used. Three interconnected nets were set at each site by placing them in the quietest part of the river in the middle or along the edge of the depression (hole), depending on the flow rate and water vortex. The nets should always be positioned parallel to the river flow (shore). An anchor was attached to the initial part of the net (located upstream) on the lower side, and a plastic buoy on the upper side (Figure 72, Figure 73, Figure 74). At some sites with higher water velocity, the net should be additionally fixed to the coast so it would not be carried downstream. The end of the net is either just connected to the buoy or in the case of a stronger water flow it is weighted by 0.5 kg heavy lead (Note: The 0.5 kg weight was used in research in Croatia and was adapted to the conditions during that part of research. The weight should be adapted to the water flow intensity of the river). During the setting of nets the water level was lower to medium and the water temperature was between 7-8 °C (the research was conducted in continental part of Croatia).

The nets should always be set in the morning and left in the water for a maximum of 4 - 5 hours. The importance for the shorter setting of nets during the day is to avoid injuries and dying of migratory species (especially Sterlet) in the nets if they are in the net for a long time as well as to avoid potential problems with poaching.

During the higher water level and the very strong water flow it is difficult to set the nets properly, which affects the positive catch results. Due to the unsuccessful result, as well as to safety reasons, the high water level and very strong water flow should be avoided for the application of this method.

Figure 72 Setting the nets with anchor and buoy at the beginning

Figure 73 Setting the nets with buoy at the end

Figure 74 Beginning of the nets and pulling the nets out of the water

Example from Croatia: The nets were set on the Sava River, downstream from Zagreb at three localities and near Jasenovac at two localities. On Prevlaka and Dubrovčak, a total of 20 sterlet (*Acipenser ruthenus*) individuals (3 and 17 individuals) were caught from 400 to 800 mm TL (Figure 75). In addition to sterlets, two localities also recorded common barbel. In one locality *Vimba vimba* and *Chondrostoma nasus* were caught. At the locality near Jasenovac *Ballerus sapa*, *Blicca bjoerkna* and *Alburnus alburnus* were recorded.

Figure 75 The sterlet caught on the wintering habitat

4.2.3 Using the sonar to document fish in wintering habitats

Acoustic methods (i.e. sonar systems) are used both in recreational and commercial fisheries and in fisheries studies for decades (Simonds, 2005). It is a good tool for finding fish specimens or fish habitats mapping. Also it could be used for the assessment of population size. These surveys have routinely been conducted during the summer months, which offers challenges for assessing population.

In aquatic research, sonar surveying methods are becoming more common due to the improvement of sonar data processing and Geographic Information Systems (GIS) software and classification models (Andrews et al., 2020). Sonar methods are desirable as a fisheries stock assessment method because they provide a rapid remote sensing of underwater habitat, without the requirement of direct observation. In fisheries stock assessment, 2D single-beam sonar is typically used from a boat, and pelagic fish species such as Herring (i.e., Clupeidae) are targeted in the water column. More recently, stationary multi-beam sonars have also been used to monitor fish movements in narrow waterbodies, such as rivers. As an additional method, side-scan sonars produce detailed image from both sides of a vessel and are, therefore, becoming common for habitat and mussel-bed mapping (Anderews et al,. 2020).

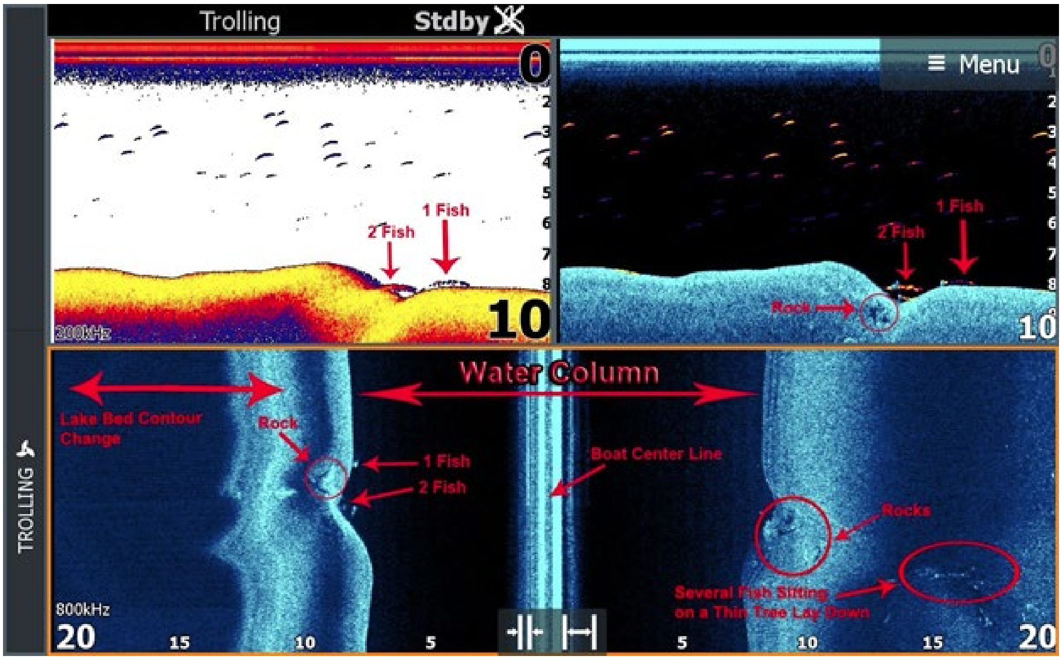

Figure 76 Example of using traditional sonar, downscan and sidescan for finding a fish (Wallace, 2020; photo credit: Lowrance and Romen Dicovski, Roaming Productions ©)

Figure 4.10.

In the sonar survey of wintering habitats the fish could be detected with the usual method of observation live on the screen of the sonar. Specific shape of the response is usually observed (Figure 76). Moreover, the species identification is one of the most challenging tasks in hydroacoustic research (Langkau, 2012; Zakharia, 1996), but the combined sonar/image processing method provides an effective tool for identification of some (larger) species (Andrews, 2020.; i.e. Shortnose sturgeon).

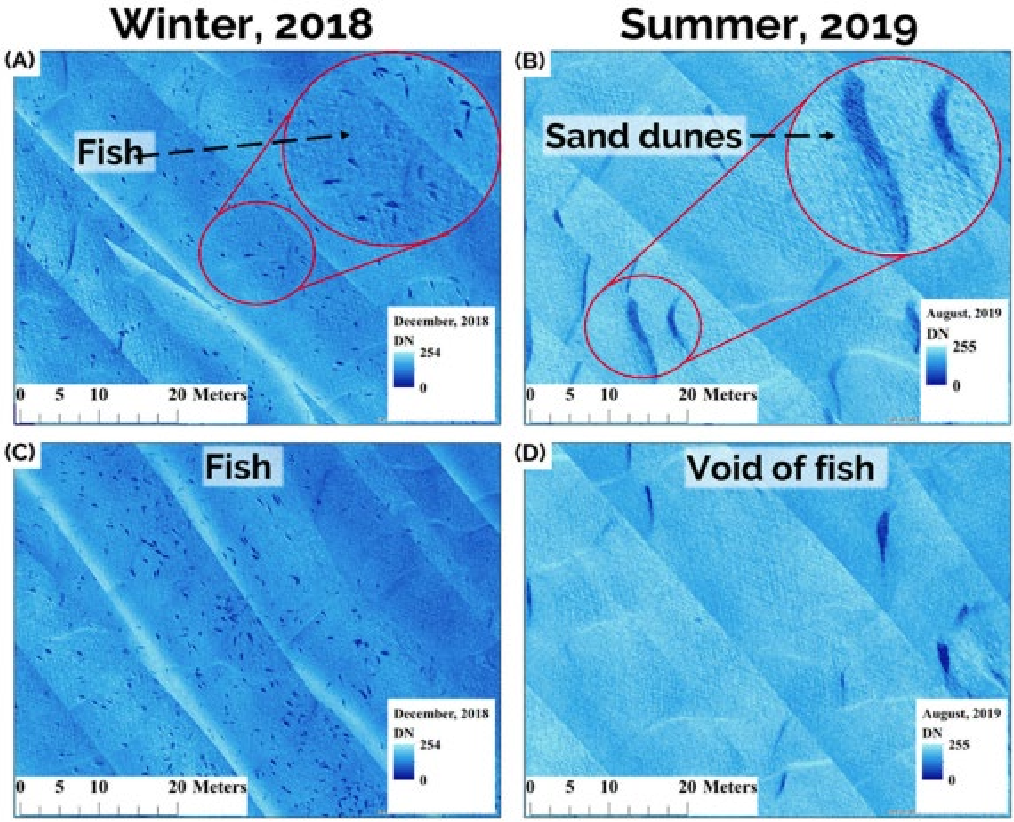

Figure 77 Example of comparison study with side scan sonar of wintering habitat scanned during the winter and summer (photo credit: Andrews et al., 2020)

Andrews et al. (2020) managed to record recreational-grade side-scan sonar, GIS, and a classification algorithm, to map and measure individuals in a previously undescribed Shortnose sturgeon wintering location (more than 12,000 individuals of Shortnose sturgeon in a single key winter location and estimated the full river population as > 20,000 individuals in winter aggregations) (Figure 77). Those side-scan sonar surveys were conducted using a commercially available Humminbird® Helix 10 MEGA SI fish finder. Side-scan sonar tracks were recorded at a frequency of 1275 kHz (Humminbird MEGA imaging®) at a ping rate of 26.1 pings per second and a scanning range of 26 m to either side of the survey vessel. Survey speed varied from 7 –10 km/h during transects and each survey was conducted as one continuous logged track. These sonar images were used along with a supervised classification model to (a) distinguish Shortnose sturgeon from the river bed, and (b) quantify Shortnose sturgeon in this area of interest. During surveys, transects were started upstream and to one side of the Sturgeon aggregation (so that sturgeon were only visible on one side of the sonar screen) and transects were continued downstream until Sturgeon were no longer visualized on sonar.

4.3. Spawning habitats

4.3.1 Capturing sturgeon on spawning habitats on large rivers (Danube)

Fishing activity of adult fish is done in special areas, known as "bottle-necks" where the fish are constrained to follow a certain itinerary to reach the spwaning area.
On the main channel, where the river is up to 1 km wide (Figure 78), the fishermen use 2 – 300 m drifting trammel nets, with 60 – 110 mm mesh size (depending on the species which is being fished) (Figure 79). The fishing team normally consists of four fishermen and two fishing boats, which fish constantly, one at a time. The fishing area is cleaned every year, mostly in spring, when the high level of water moves a lot of trees.

Figure 78 Fishing activity in the main channel (photo credit: Marian Paraschiv)

Figure 79 Lifting the drifting trammel net used for capturing adult sturgeons (photo credit: Marian Paraschiv)

Every caught fish is gently lifted from the water and from the net, general health condition is verified and then is realeased back in the river as soon as possible and preferably in the same location where the fish was captured (Figure 80).

Figure 80 Releasing one adult Stellate sturgeon back in the river (photo credit: Marian Paraschiv)

Every caught fish has his own record sheet where ll data are recorded, including individual photos. Taking photos is the best way to recognize a particular fish and to have good information about different phenotypes (Figure 81), but also to have a good picture to learn to recognize different species (Figure 82).

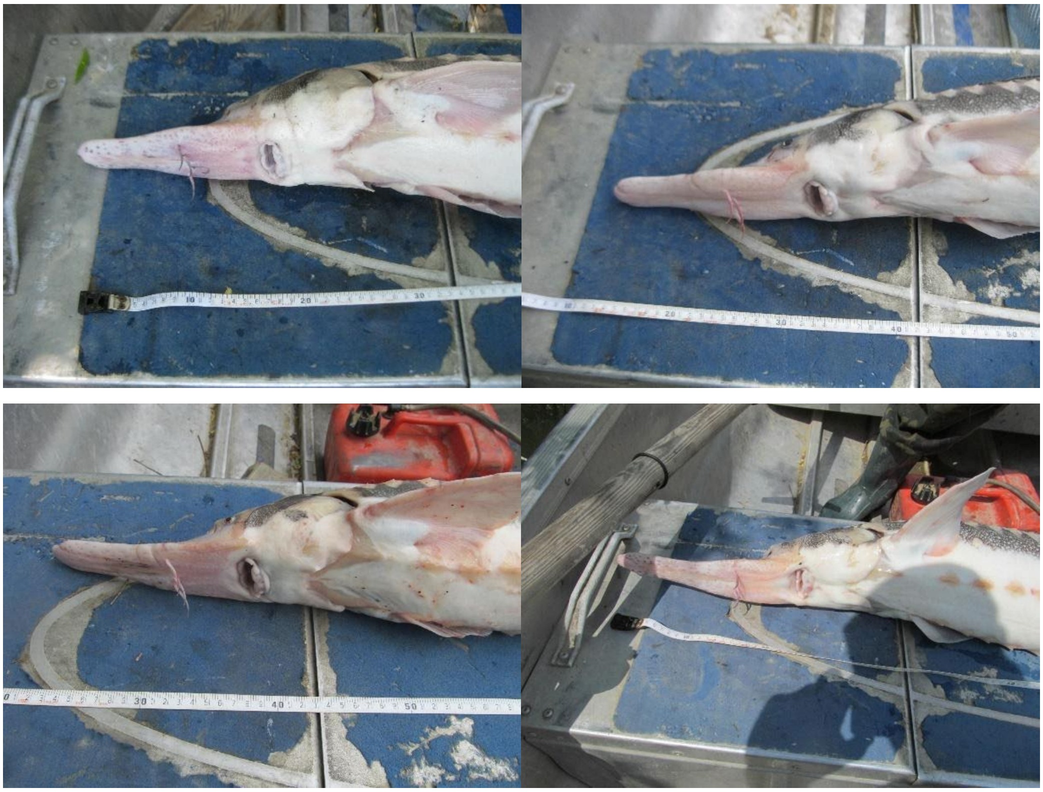

Figure 81 Different phenotypes of wild Stellate sturgeons (photo credit: Marian Paraschiv)

Figure 82 Adult Stellate and Russian sturgeons (photo credit: Marian Paraschiv)

4.3.2. Capturing sturgeon larvae downstream of spawning habitats in the Danube River

To confirm sturgeon habitats in the Danube River habitat, one of the best method is to capture early life stages (ELS) – drifting eggs, free embryos and feeding larvae downstream (very close) of the sturgeons spawning habitats.
The fishing tool used to capture on the bottom of the river ELS of sturgeons and other benthic fish is the D-shaped drift net for eggs and larvae, manufactured by Duluth Fish Nets, Minnesota, USA.
The net (Figure 83) is 4.5 m long with the largest opening of semi-circular shape / D-shape with 1 m diameter. The metal frame was manufactured by us of 8 mm diameter steel bar, adding on the diameter of the frame a consistent lead weight by inserting the linear part of the frame in a mould made of thin metal sheet, in which we pored melted lead to form bar of about 40 mm diameter. This weight is meant to ensure correct positioning on the bottom of the river when the net is installed for fishing. Net mesh size is 2 mm and diameter of the nylon threads is 1 mm. Newer versions do not use any more nylon netting but some new, knotless netting of extremely resistant synthetic material.

Figure 83 D-shape net ready to be installed at Danube River Km 311,

The most appropriate place to install the D-shape net is on the main channel, where the current is strong and is moving eggs and larvae downstream from spawning places. The net stays in the water over the night and is lifted every 12 hours (sometimes even more often, depending onthe water current which transports many debris and could fill the net), to check the content.
Every ELS caught is preserved in 99.99% alcohol and by using genetic methods, the species is very precisely determined in the lab. Since 2000, DDNI investigates two spawning habitats for sturgeons by fishing ELS (Isaccea – R Km 100 and Rasova – R Km. 310), where many larvae were captured (Figure 84**)**.

Figure 84 Beluga sturgeon (Huso huso) larvae and Sterlet (Acipenser ruthenus)

4.3.3. Using e-DNA to locate spawning activity

Environmental DNA (eDNA) refers to the amplification of DNA directly from the environment without first being isolated from any target organism. Its main aim is to determine the presence/absence or quantity of single or few species (Environmental DNA "*sensu stricto*"). The recent wider application of eDNA studies is linked with the increased Next Generation Sequencing techniques. Environmental DNA "*sensu lato*" incorporates both metabarcoding and metagenomics and applications include dietary analysis, the study of food webs interactions, microbial ecology, changes in species distributions, the stability of the niche, and assorted biodiversity research (Rowe et al., 2017).

Analyses of eDNA in the freshwater ecosystems are rapidly increasing and are used for fish biodiversity assessment (Goutte et al., 2020; Lecaudey et al., 2019), monitoring of invasive species (Guan et al., 2019), fish abundance and biomass (Doi et al., 2017; Pont et al., 2018) or evaluation of ecological status (Hering et al., 2018). The recent improvements to quantify the data from eDNA analysis enable studying the spawning behaviour of fish species.

Most of the fishes have external fertilisation, so reproductive cells are massively released to the water. Additionally, spawning is coupled with higher activity e.g. physical contact during spawning or preparation of spawning nests. Such changes in the amount of genetic material are well reflected in the eDNA samples that were also confirmed in laboratory conditions (Bylemans et al., 2017). Several studies dealing with the spawning behaviour were done in previous years (Erickson et al., 2016; Thalinger et al., 2019).

4.3.4 Visual observation of spawning activity

Counts conducted from stream banks are simple to perform and require only a modest amount of equipment (Zale, Parrish & Sutton, 2012). This method can be successfully implemented to confirm possible spawning locations by recording spawning activity of fish species that spawn either in shallow water, like the Black

Figure 85 Spawning activity of Nase (Chondrostoma nasus) in the Sava river in Slovenia (photo credit REVIVO 2019).

Figure 86 Spawning activity of Nase (Chondrostoma nasus) in the Sava river in Slovenia (photo credit: REVIVO 2019).

Sea trout (*Salmo labrax*), Barbel (*Barbus barbus*), Nase (*Chondrostoma nasus*) (Figure 85 & Figure 86), Vimba bream (*Vimba vimba*), Cactus roach (*Rutilus virgo*), Asp (*Aspius aspius*) and Danube salmon (*Hucho hucho*) or in heavily vegetated areas, like the Common carp (*Cyprinus carpio*). To observe possible spawning activity from the bank of a watercourse, one or several observers should slowly and silently approach the water edge, taking care not to startle any fish in the vicinity. Polarized sunglasses should be worn, which enable the observer to better identify what is happening below the water surface. Signs of spawning activity are energetic swimming, thrashing and the accompanying splashes of water, caused by the spawning fish. Fish identification or quantification can be done with eye observation, sampling with nets or with electro-fishing, however we do not recommend electro-fishing during spawning activity unless crucial for the research.

4.4. Nursery habitat

4.4.1.Fishing young of the year (YOY) sturgeons at nursery habitat

YOY fishing should be conducted to capture recruits, which in case of LDR sturgeons are YOY with TL≥10 cm, having an estimated survival rate of over 90 % to the age of first sexual maturation to return to the river for spawning. In anadromous species (*H. huso, A. stellatus* and *A. gueldenstaedtii*) at this body length the YOY have fully developed body scutes with sharp tips and warning coloration of body and scutes (Figure 87).

Figure 87 : YOY Huso huso (left) (29.06.2006), YOY and age 1+ Acipenser ruthenus (right) (24.07.2009), allcaptured at Danube km 123 (photo credits: Marian Paraschiv / DDNI Tulcea)

DDNI Sturgeon Research Group (SRG) starts the monitoring of annual recruitment by capturing YOY at nursery area since year 2000, using different information from national authorities and data collected on the field. The arrival date of the first YOY sturgeons at the nursery habitat of Danube km 123 (best known nursery place in LDR) was estimated using the daily water temperatures recorded and posted on webpage of AFDJ Galați https://www.afdj.ro/ro/cotele-dunarii.

For fishing YOY sturgeons a trammel net (Figure 88) with18 – 20 mm mesh size is used, adapted to the width of the river and tuned to work in contact with the ground, drifting on the bottom of the river.
Trammel nets with float lines and lead lines incorporated in the ropes are the best / easy to deploy and to lift (also, to avoid entangling of the net).

Figure 88 Aluminium boat and fishing crew with trammel net at D m 123 (June 22, 2009) (photo credit: Elena Taflan / DDNI Tulcea)

To be comparable, the abundance data of capturing YOY sturgeons in each year should be expressed in Catch Per Unit of Effort **(CPUE).** This is the total number of specimens of one species captured in that year divided by the total number of trammel netting / hauls (in Romanian – toane; in Ukrainian - tonya) in which the fish were captured.
These data can be presented in bar charts also called recruitment indices or juvenile production indices (JPI).
Bellow we provide examples (Figure 89, Figure 90, Figure 91, Figure 92) of JPI of YOY sturgeons monitored by the DDNI SRG team over a period of 20 years (2000 - 2019) by trammel netting in the LDR, at Danube River km 123.

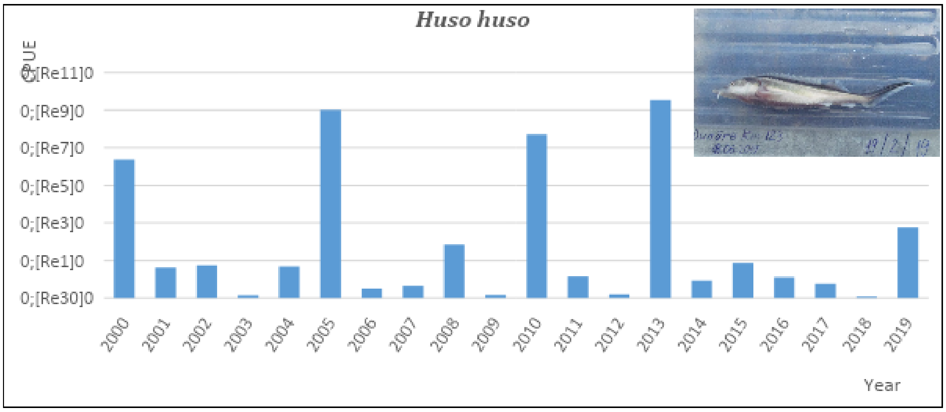

Figure 89 Abundance of YOY Beluga sturgeons (Huso huso) during 2000 – 2019 at nursery site located of D km 123 (Report DDNI 2019)

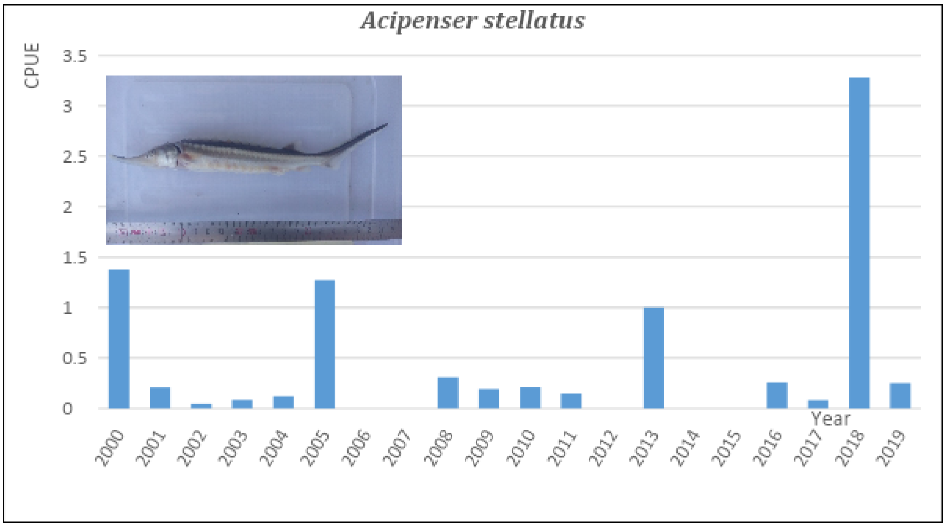

Figure 90 Abundance of YOY Stellate sturgeons (A. stellatus) during 2000 – 2019 at nursery site located ofD km 123 (Report DDNI 2019)

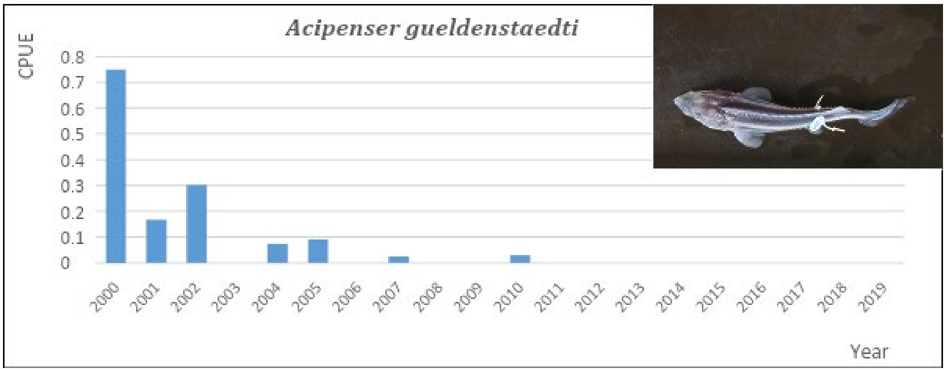

Figure 91 Abundance of YOY Russian sturgeons (A. gueldenstaedtii) during 2000 – 2019 at nursery site located ofD km 123 (Report DDNI 2019)

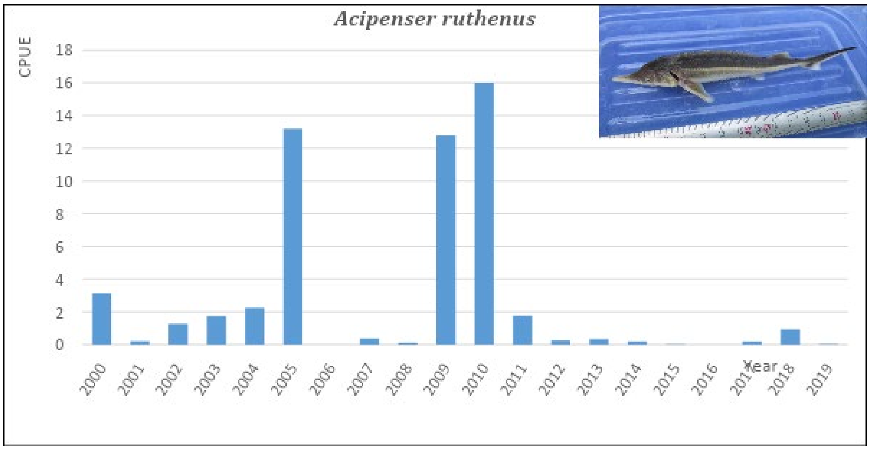

Figure 92 Abundance of YOY Sterlets (A. ruthenus) during 2000 – 2019 at nursery site located of D km 123 (Report DDNI 2019)

4.5. Feeding habitats in the river and in the sea

4.5.1 Feeding habitat in the Danube River

Fish feeding habitats have a particular importance due to the information they can provide by analysing the food types consumed by the studied fish captured in the feeding area during the feeding period time. This can be done by investigating the most frequently consumed prey to understand feeding behaviour and food types preferred by fish and to quantify the ingestion rate of individuals of different life stage. The stomach content data collected also helps to better understand many aspects of fish ecology at population level or individual level by providing information regarding fish interaction with the prey, examine dead fish in the context of fish community, existence of competition for food, the feeding periodicity and to estimate the amount of consumed food at the individual and population level (Allen, 1951; Staples, 1975). As a result, stomach content studies due to the information that may

be collected, could be incorporated into a variety of different research objectives (Mahesh, 2018).
To confirm the feeding habitats, the relationship between the bentofauna available in the river and the food in the stomach of fish must be analyzed.
Bentofauna sampling is performed at km 123, in the area of the Romanian bank of the Danube, using a Van Veen dredge, with a working surface of 0.05 m².
Bottom sample collected from the feeding habitat located at rkm 123, using a Van Veen grab sampler in 2018 reveal presence of more invertebrates species besides the *Tubifex* species and *Gammarus* species that are most commonly found in the stomach sturgeon species captured in the feeding area. For the bottom fauna assessment there were 9 samples of benthos taken from the beginning, middle and end of the feeding area, to have an overview of the benthofauna present at the feeding habitat (Table 2)

Table 2 Example of invertebrates found in each sample collected in the feeding habitat located in Danube River at km 12

Sample name	Sample location Latitude	Sample location Longitude	Date	Water depth [m]	Tubifex tubifex	Gammarus	Lithogliphus naticoides	Dreissena polymorpha	Unio pictorum	Corbicula fulminea	Unio tumidus	Anodonta cygnea
				Number of invertebrates found in each sample								
A1	450 24,421`	280 16,861`	11.09.2018	**4.9**	**3**	**2**	**46**		**1**	**2**	**4**	
A2	450 24,395`	280 16,922`	11.10. 2018	**9.4**	**24**		**5**			**1**		
A3	450 24,403`	280 17,016`	11.10. 2018	**13.9**	**6**	**2**	**4**			**2**		
B1	450 24,219`	280 16,849`	11.10. 2018	**4.6**			**27**			**2**		
B2	450 24,207`	280 16,903`	11.10. 2018	**7.5**	**1**	**6**	**16**			**4**		
B3	450 24,246`	280 17,024`	11.10. 2018	**13.8**	**10**	**2**	**8**				**1**	
C1	450 24,067`	280 16,833`	11.10. 2018	**4.4**	**8**		**95**				**1**	
C2	450 24,036`	280 16,917`	11.10. 2018	**9.1**	**106**	**1**	**5**					
C3	450 24,043`	280 17,017`	11.10. 2018	**14.4**	**8**		**10**			**2**		
D1	450 23,784`	280 16,866`	11.10. 2018	**4**	**122**		**162**	**1**	**1**		**4**	
D2	450 23,786`	280 16,947`	11.10. 2018	**12.4**	**118**		**137**	**2**		**7**		**2**
D3	450 23,848`	280 17,029`	11.10. 2018	**13.6**	**9**		**12**			**7**		

Analysing the food abundance (Danube River Km. 123) by years with theVan Veen dredge, it was found that the maximum density of specimens varies between 7 - 800 ex. /0.05 m² in the summer of 2003 and 2009 and 565 ex. / 0.05 m² on June 15, 2018. Even in the case of samples taken on October 11, 2018, when the water temperature was lower by almost 10 °C, it is observed that the maximum number of *Tubifex sp.* exceeded the value of 100 ex. / 0.05 m² (Paraschiv et al., 2018).

The stomach content can be extracted from dead fish using a surgical method, but there are also non traumatizing methods that can be used in order to keep the fish unharmed and to release it after the extraction procedure is done. One non-traumatizing method used to analyse sturgeon stomach content captured in the Danube River or the Black Sea was performed using a syringe and small tubes, suitable to extract ingested food from the smaller fish when the extracting device from other methods is too large to be used (Baker & Fraser, 1976). The extracted samples can be analysed in the field or can be preserved in 95 % ethanol for transport and for detailed laboratory analyses (species determination, quantitative analysis, and species distribution (Paraschiv et al., 2010) (Figure 93).

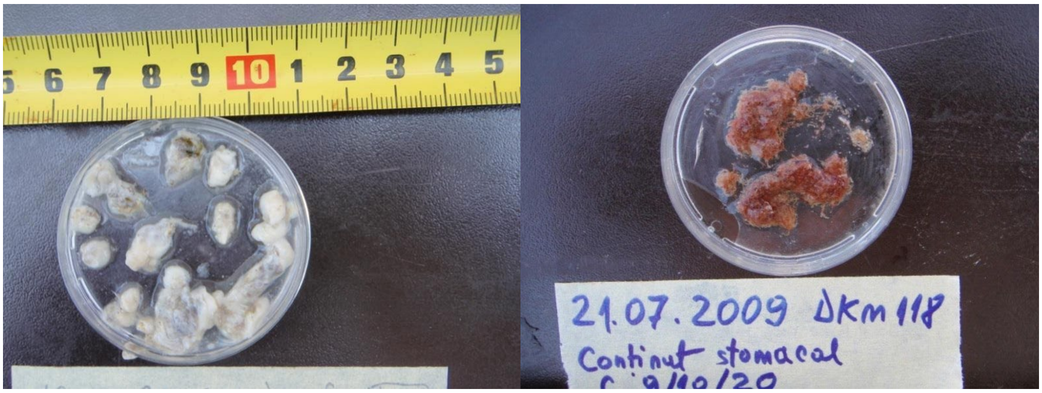

Figure 93 Stomach content extracted in 2009 from a Sterlet captured in Danube River at km 123 (undigested invertebrates – left, Tubifex- right) (photo credit: Marian Paraschiv)

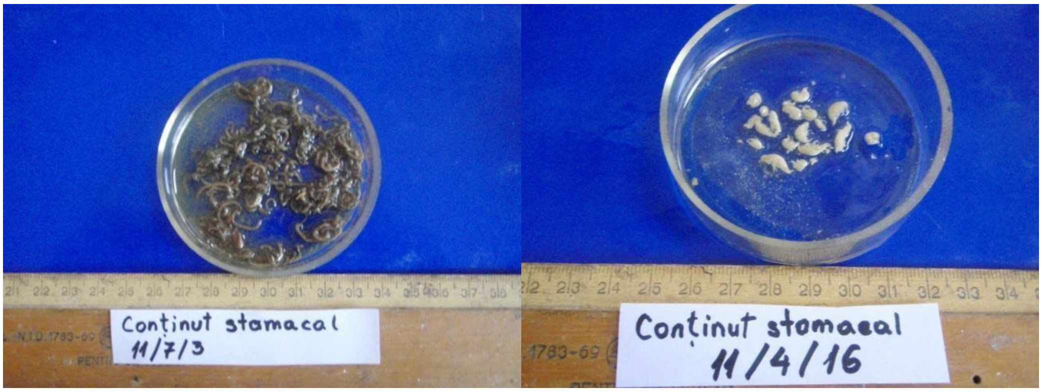

Figure 94 Stomach content samples extracted in 2011 from a Sterlet (left) and Beluga sturgeon (right)

Stomach samples extraction from juvenile sturgeons was performed usingstomach flushing, or gastric lavage method which is a widely used technique in live fish to obtain the stomach contents (Hyslop, 1980) (Figure 94). There are several different techniques and various types of equipment that are used to flush out the stomach contents of fish. Stomach flushing equipment has been categorized under hand pumps, mechanized pressure, or syringes, although there is some overlap with these groupings (Kamler & Pope, 2001; Seaburg, 1957).

4.5.2 Feeding habitat in the Black Sea coast

Similar methods were used to extract stomach content from the sturgeons captured in the feeding habitats located in the Black Sea at the mouth of the St. George branch in order to asses stomach content of the juvenile sturgeons stationed in the shallow waters of the sea (Cristea et al., 2016). Most of the specimen captured in the feeding area from the Black Sea was Beluga sturgeon, Russian sturgeon and Stellate sturgeon (Figure 95). A large part of the specimens captured were sturgeon that were restocked in the river in the previous year (2013) and stopped in the feeding area together with the wild sturgeon specimens to feed before migrating in the deeper waters of the Black Sea. The project that also has restocking success assessment component revealed that the restocked sturgeons / farm sturgeons have no problem to adapt their feeding behaviour to any wild type of food, in the moment they were in the Danube River and the Black Sea after they were kept captive into a farm for more than 1 year (where they were fed artificial food / fish pellets food) (Cristea et al., 2016).

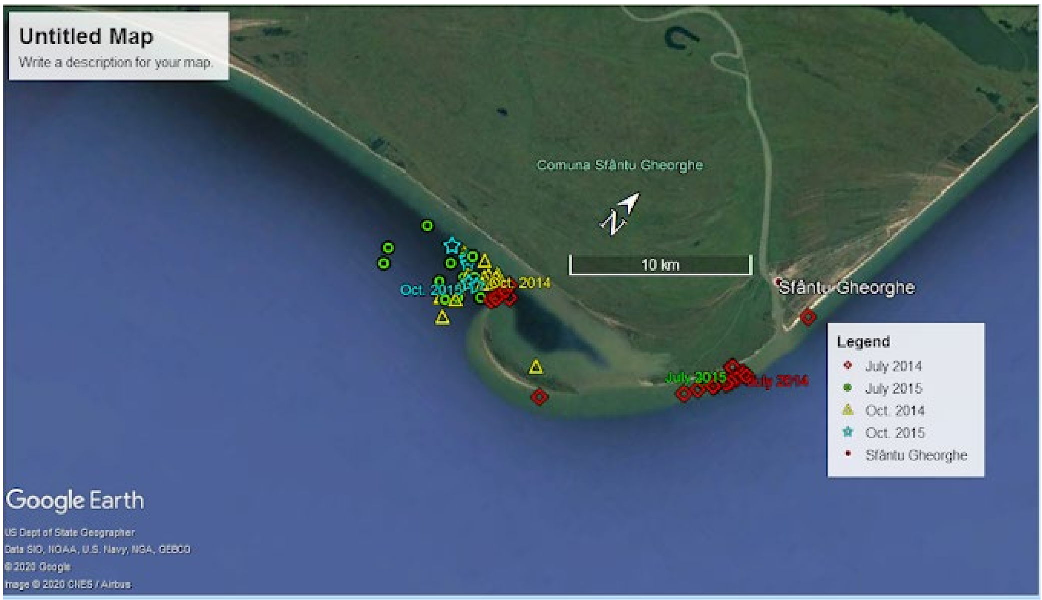

Figure 95 Capture locations of Beluga, Stellate and Russian sturgeon juvenile specimens in the feeding area of the Black Sea coast (photo credit: Cristea et al., 2016)

It can be observed how the sturgeon's eating habits evolve once they arrive in the Black Sea and become larger fish. There are new and larger species of fish and of invertebrates found in their stomach. The stomach content of the juvenile sturgeon species captured in the Black Sea feeding area reveal presence of small fish and other shellfish species (Figure 96).

Figure 96 Stomach content of 2 Beluga sturgeon juvenile captured in the Black Sea feeding area (up left & right); Beluga sturgeon Juvenile (down) captured in the Black Sea feeding area (project POP 3.5 UDJ Galati) (photo credit: Cristea et al., 2016)

4.6. Habitat confirming by tagging of fish

4.6.1 Tagging of adult fish

Tagging techniques of the fish in the rivers are relativelly new, being used in thepast mostly for tag migratory fish from oceans and seas. Most of the tags reveal with accuracy the position of the fish, helping in fish habitat identification.

The difference between tag and mark is subtile and at first sight imperceptible. A mark is an external, internal or embedded device in the skin that aims to recognize the individual. A tag is usually attached externally or internally and contains specific identification information (Jones, 1979). Despite these differences, the tag name is usually used for both types of devices. The tagging of fish was first used a few hundred years ago. For example, Izaak Walton states that in 1653 brook guards attached coloured cords to the tails of Atlantic salmon (McFarlane et al., 1990). In the past years, tagging technologies have seen unprecedented development, especially in order to reduce the size of devices as well as to reduce the side effects.

In general, three categories of data are obtained through marking and tagging studies:

a) marking individuals for specific activities (e.g. in aquaculture, to know the history of individuals);

b) marking individuals allows their identification when moving or mixing with individuals from other populations;

c) marking offers the possibility to collect data that can be statistically processed (e.g. estimating the abundance of a population, estimating natural mortality, etc.

Premises of using marking techniques

The first and most important aspect is that a tagged fish must be easily recognizable and the process must be as stress-free as possible. Two main aspects need to be considered: tag retention should be as close to 100 % and each tagged fish can be easy to identify and report. Retention of the tag can be influenced by different factors: the type of tag (size, shape), the location of the attaching and the species to be studied.

In the Danube River DDNI developed few tagging techniques and devices for adult sturgeons:

1. Tagging (cutting) of the fins

This method of cutting or perforating fins has been used since the 1800s and is accepted by most specialists as the most widely used marking method (Figure 97). The process is simple and fast, but identifying individuals is limited because only a few punching/cutting combinations can be used.

Figure 97 Tagging of Acipenser stellatus by cutting of anal fin (photo credit: M. Paraschiv)

2. External tags with "arrow" and "T" type attachment

These types of external tags are the most widely used and are mainly made of plastic or thin wire, usually penetrating only one side of the fish's body (Figure 98). The end of the mark with which it is attached to the body of the fish is in the shape of an arrow or in the shape of a letter T. In general, these marks attach just below the dorsal fin, in the white muscle group, which has few blood vessels and therefore does not cause heavy bleeding.

3. Satellite tags (PSAT or PAT tag)

A satellite tag is generally constructed of several components: a data-logging section, a release section, a float, and an antenna. The release sections include an energetically popped off release section or a corrosive pin that is actively corroded on a present date or after a specified period of time. A PSAT (or PAT tag) is an archival tag (or data logger) that is equipped with device to transmit the collected data via the Argos satellite system. Location, depth, temperature, oxygen levels, and body movement data are used to answer questions about migratory patterns, habitat movements, daily habits, etc. DDNI have been using this system (MK 10) since 2009 to track movements of adult Beluga sturgeon (*Huso huso*) in the Dnube River and in the Black Sea. Four Beluga males were tagged with PAT Mark10 system (Figure 99) and were released in the Danube river, on the Borcea branch.

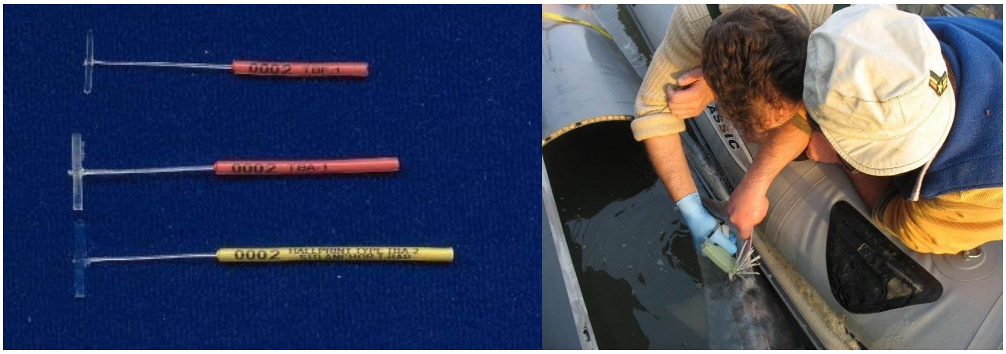

Figure 98 Left – External T tags; Right – the method of attaching the tag to a Huso huso male)

Figure 99 PAT MK 10 tag attached on Huso huso male

Figure 4.33:

4. PIT tags (Passive Integrated Transponder)

A PIT tag consists of an integrated circuit chip, capacitor, and antenna coil encased in glass (Roussel et al*.,* 2000). PIT tags vary in size and shape depending on the study animal. Generally, tags are cylindrical in shape, about 8 - 32 mm long, and 1- 4 mm in diameter.

Essentially, PIT tags act as a lifetime barcode for an individual animal, analogous to a social security number and, provided they can be scanned, are as reliable as a fingerprint (Gibbons & Andrews, 2004). PIT tags are dormant until activated; they therefore do not require any internal source of power throughout their lifespan. To activate the tag, a low-frequency radio signal is emitted by a scanning device that generates a close-range electromagnetic field. The tag then sends a unique alpha-numeric code back to the reader (Keck, 1994). Scanners are available as handheld, portable, battery-powered models and as stationary, automated models that are usually used for automated scanning.

Internal PIT tags are inserted via large-gauge needles or surgically implanted either subcutaneously (Figure 100) or into a body cavity. To check the ID fish, an external PIT detector is needed (Figure 101).

Figure 100 Implanting PIT tag to Acipenser stellatus (photo credit: Marian Iani)

Figure 101 Checking the fish ID using PIT detector (photo credit: Marian Iani)

5. Acoustic (ultrasonic) tags

Acoustic tags are small sound-emitting devices that allow the detection and/or remote tracking of organisms in aquatic ecosystems. Acoustic tags are commonly used to monitor and track fish movements, being implanted in the fish body cavity. The signal – "pings" propagation is up to 1 km, depending of the water turbidity, the water current or river bottom structure. The detection of the acoustic tagged fish is made by using acoustic receivers installed in a different network (Figure 102 A), to cover all the study area. DDNI have been using this tagging method since 1997 because it is a reliable method and allows the observation of the behaviour of fish for a long period. To implant the tag (Fig. 4.36 A), a scalpel is used to make a 5 - 6 cm incision through which an acoustic emitter is inserted into the abdominal cavity. After this operation, the incision is sewn with surgical thread over which a surgical adhesive is applied (Figure 102 B).

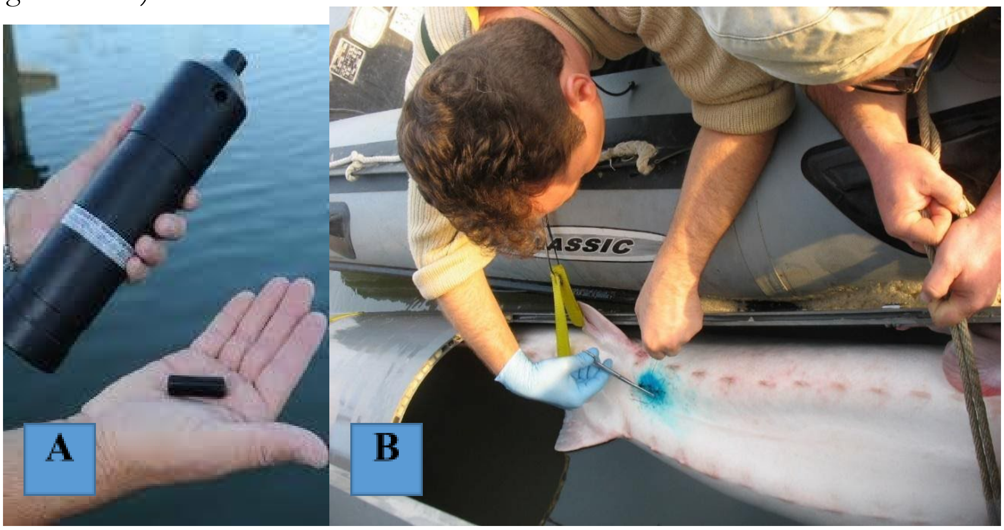

Figure 102 A – Acoustic tags and acoustic receivers used by DDNI to tag adult sturgeons (source: www.vemco.com) B – Incision made to insert the acoustic tag in the body cavity of a Huso husomale (photo credit: Marian Paraschiv)

4.6.2 Tagging of young (YOY) fish

The tagging technique of young fish involve pretty much the same procedure like adult fish, but due to their sensibility to handling, all the actions must be conducted quick and precise:

1) Hold fish in aerated holding tank(s) (5 – 50 l), depending onnumber and size of the fish (Figure 103)

2) Biometric measurements (TW, TL) and sample fin clips in 96 % ethanol

Usually, the measurements are made in an etalon box, to have standardized measures and to be able to use the photos in the statistical analyses using different software (Figure 104)

Figure 103 Holding fish in 50 l aeratedholding tank.

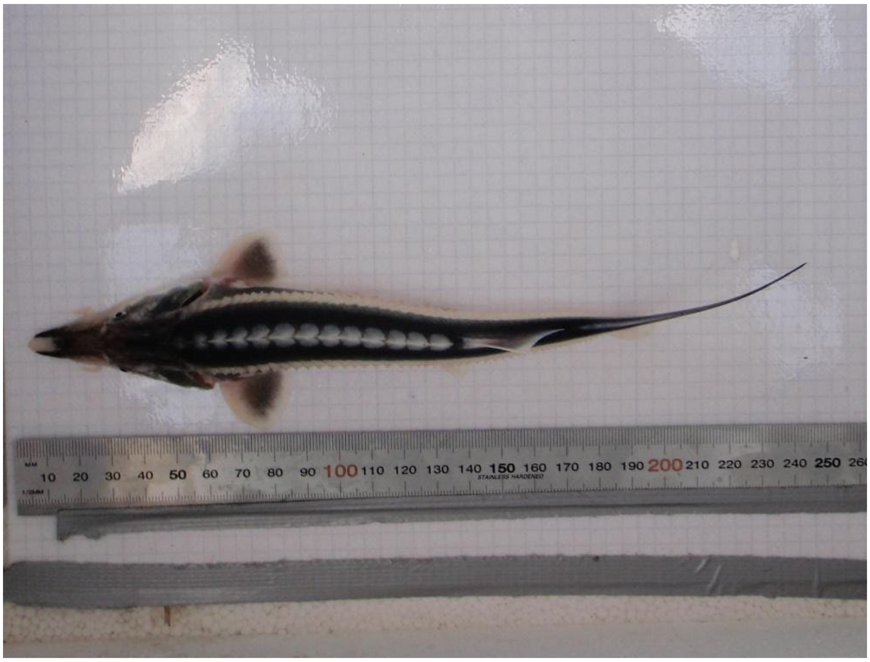

Figure 104 Measuring Beluga YOY

In order to collect fin samples, the fin is wiped with 70˚ alcohol and a fragment of approx. 1 cm^2 is cut (Figure 105) and quickly inserted into the previously marked Eppendorf tube (1.5ml), containing 99 % alcohol concentration. Take appropriate photos of fish in dorsal, lateral and ventral view (include date and site identification label).

Figure 105 Collection procedure for genetic analysis / DNA of the anal fin fragment

3) Tagging YOY

In order to study YOY behaviour in the monitoring area, all captured YOY are marked with differnt tags(Figure 106, Figure 107, Figure 108).

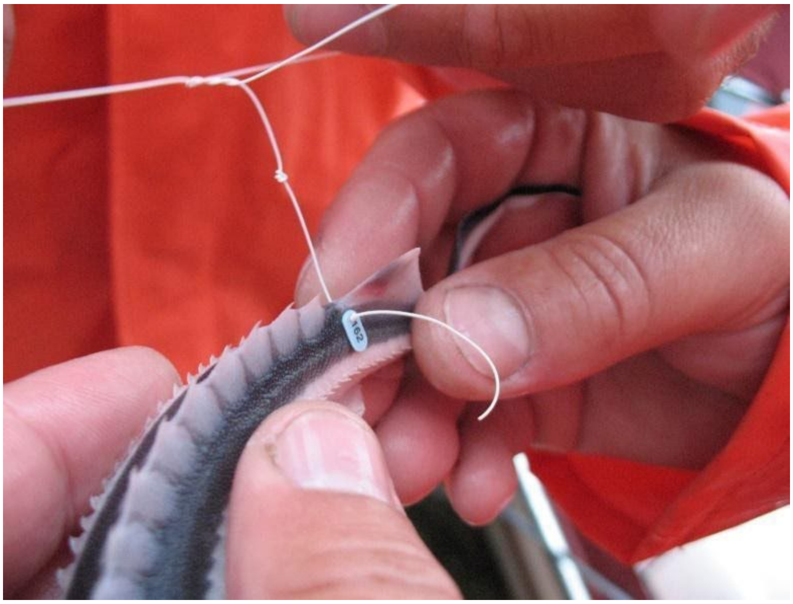

Figure 106 FFT tags attaching procedure

Figure 107 Implanting acoustic tag (left) and Beluga sturgeon tagged with VP13 tag (right)

Figure 108 One year old Russian sturgeon tagged with T-bar

4) Taking photos of live YOY – to have good photos, sturgeons can be kept out of the water / in air for 1-2 minutes to weaken and reduce their motility. For every fish we have to take at least two photos – from dorsal and side / lateral view (Figure 109)

Figure 109 Dorsal and side photos taken at YOY Beluga in 2019

5) Releasing the fish into its natural environment

All fish that are captured in the monitoring activity should be released back in the natural environment. Since all the measures are time consuming, the fish could be tired / stressed an have to be put in the holding aerated tank for few minutes, for recovering. When it is clear that the fish are in good condition and swim continuously, the fish are released back in the river (Figure 110), preferably in the same place where they were caught.

Figure 110 Releasing the fish back in the river

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CHAPTER 5

Advantages, limitations and estimated costs of different methods used in migratory fish habitat assessment

Overview

In this chapter, we summaries all the methods described in the previous chapters in two tables. The methods are ordered into 18 groups: substrate identification, water velocity, discharge dynamics, water depth, water temperature, turbidity, MZB abundance and composition, using cartographic materials to identify potential key-habitats, mapping riverbed morphology, capturing adults, using electro-narcosis, tracking movements using telemetry to demonstrate habitat use, capturing eggs and larvae of benthic fish (D-shaped drift net), capturing pelagic eggs and larvae of shads (Alosa sp.), capturing YOY sturgeons, monitoring annual recruitment of sturgeons, sampling YOY and tracking movement and nursery habitat use by YOY (acoustic telemetry). The Table 1 presents the advantages and disadvantages of each method to aid in the determination of the methods adequacy in other research applications and gives references for each method described. The Table 2details all of the equipment necessary for the implementation of each of the methods, the approximate costs of each individual piece of equipment, the annual costs of operating said equipment and the personnel and level of training required to implement the methods successfully.

Table 3 Advantages and limitations of different methods.

Method	Advantage(s)	Limitation(s)	Source
1.**Substrate identification:**			
1.1 Kynard probe	Cheap and easy to construct, easy to handle and assess the type of substrate.	Rough but still enough precision of measuring the size and locating the suitable hard substrate if used in combination with handheld GPS; requires	Written by REVIVO, Source: Kynard, B., Zhuang, P., Zhang T., Zhang, L. (2002). Ontogenetic behavior and migration of Volga River Russian sturgeon, Acipenser gueldenstaedtii, with a note on adaptive significance of body color. Environmental Biology of Fishes, 65: 411 – 421

		boat and at least two operators.	
1.2 Side scanner	Used properly it can produce maps with detailed positioning of reaches of the river bottom with suitable spawning substrate at any water depth condition in the Danube and tributaries.	Expensive as specialized software is needed. Instead of expensive professional software, inexpensive fish finders/sonars can be used. Requires boat and trained professionals.	Written by REVIVO, Source: Kaeser A.D., Litts T.L., Wesley T., T. (2012). Using Low-Cost Side-Scan Sonar For Benthic Mapping Throughout The Lower Flint River, Georgia, Usa. River Research and Applications.
1.3 Van Veen	Very efficient, as it allows for simultaneous sampling of sediment and macroinvertebrates.	Not suitable for hard or extremely soft top layers of sediment.	Written by REVIVO, Source: 2018: Van Veen grabs Manual, Eijkelkamp Soil & Water. 4p.
1.4. Water Wolf camera	Simple, cheap and requiring minimal equipment.	Transparency and flow dependent. Requires boat and at least two trained operators.	Written and tested by REVIVO
2. **Water velocity:**			
2.1 Electro-magnetic flowmeter	With sensors attached to a gauging weight suspended on a cable of a centimetric winch it can provide very accurate velocity measurements at any depth in the DR and tributaries.	Moderate cost; requires boat with anchoring system; beware of direction mistakes in eddies.	Written by DDNI, Source: Hont S., et al. (2018). Review & Completion of the Feasibility Study for the Improvement of Navigation along the Joint Danube Romanian - Bulgarian Sector & Complementary Studies “FASTDANUBE”, Consultancy Contract 965 HRO
2.2 ADCP	Relatively simple to	Expensive; requires boat	Written by DDNI,

	operate; provides accurate cross profiles which are automatically recorded and can be studied in the Lab.; easy to detect areas of counter-current (eddies); provides good discharge estimation.	and at least two staff members; does not measure velocities in 10% of the water column above the ground of the riverbed.	Source: Turnipseed, D.P., and Sauer, V.B., (2010). Discharge measurements at gaging stations: U.S. Geological Survey Techniques and Methods book 3, chap. A8, 87 p. (Also available at https://pubs.usgs.gov/tm/tm3-a8/.)
3.**Discharge dynamics**			
3.1 Water level records by navigation authority	Easy to access on the internet, usually displaying measurements in all major ports on the river.	Sometimes the gauging station is located far from the reach of interest and values require certain extrapolation.	Written by DDNI, Source: https://www.afdj.ro/en/content/danube-water-level
3.2 Discharge recorded by regional water management authorities	Very useful in retroactive studies when assessing spawning conditions during a season or the whole year.	Sometimes data should be paid for.	Written by DDNI, Source: http://www.inhga.ro/diagnoza_si_prognoza_dunare
3.3 ADCP	Relatively simple to operate; provides accurate cross profiles which are automatically recorded and can be studied in the lab.	Effect of sediment on backscattered acoustic energy and bottom tracking, and unmeasured areas of a profile associated with transducer draft and ringing and side-lobe interference.	Written by DDNI, Source: Mueller, D. S., Wagner, C. R. (2009). Measuring discharge with acoustic Doppler current profilers from a moving boat: U.S. Geological Survey Techniques and Methods 3A–22, 72 p. (available online at http://pubs.water.usgs.gov/tm3a22).

4. **Water depth**			
4.1 Handheld sonar	Simple, light and easy to use; no separate car battery needed.	Beware of pointing the instrument vertically in the water.	Written by DDNI, Source: https://www.projectk.co.jp/marine/pdf/2017_Hawkeye_Catalog.pdf
4.2 Fishfinder sonar	Once properly installed on a boat it provides a wealth of information of water depth and river bottom morphology, including presence and size of fish.	Limited use in shallow water conditions.	Written by REVIVO, Source: Kaeser A. D., Litts T. I Scan Sonar For Benthic Mapping Throughout The Lower Flint River, Georgia, Usa. River Research and Applications.
5.**Water temperature**			
5.1 Multi-meter	Useful in water bodies with temperature stratification when operated attached to a gauging weight (discharge of cooling water).	Best used from a boat with anchoring system.	Written by DDNI, Source: Mingli L., Daoliang L., Qisheng D., Ya C., Chengfei G. (2013), A Multi-parameter Integrated Water Quality Sensors System, http://dx.doi.org/10.1007/978-3-642-36124-1_32
5.2 Submerged temperature recorder	Temperature mini-loggers can be deployed at any place to record at programmed timing for many months and even years; very reliable data.	Risk of vandalizing (anglers and tourists) or damaging by navigation and hydrotechnical and sand/gravel extraction works; downloading data requires laptop computer.	Written by DDNI, Source: Joanna B. W., Jacob T. W. , Craig P. P., Robin M. R., (2020). Use of Multiple Temperature Logger Models Can Alter Conclusions, Water 2020, 12, 668; doi:10.3390/w12030668
6. **Turbidity**			
6.1 Secchi Disk Visibility (SDV)	Cheap and easy to construct; easy to	Requires presence of operator personnel; best	Written by DDNI, Source:

	handle and read SDV.	used / requires use of boat.	Leszek A. B., (2009). Secchi disk, Chapter: Encyclopedia of Earth. Eds. Cutler J. Cleveland (Washington, D.C.: Environmental Information Coalition, National Council for Science and the Environment). [First published in the Encyclopedia of Earth October 15, 2009]
6.2 Turbidimeter (data logging)	Programmable; data logging; even remote data transmission in some type.	Expensive; risk of vandalizing and loosing recorded data.	Written by DDNI, Source: Kazuhisa A. C., (2012). The Intra-Annual Variability of Discharge, Sediment Load and Chemical Flux from the Monitoring: The Yukon River, Alaska, DOI: 10.4236 [illegible] Protection 04(04):173
7.**MZB abundance and composition**			
7.1 MZB -1 by stationary drift net	Can collect drifting macro invertebrates from hard substrates; used with flowmeter can provide abundance / m^3.	In deep rivers requires use of boat, floats, ropes and anchor(s); risk of vandalizing if left unguarded.	Written by REVIVO, Source: Graf W., Csányi B., Leitner P., Paunović M., Huber T., Szekeres J., Nagy C., Borza P. (2014). Full report on: Macroinvertebrates, Joint Danube Survey 3. ICPDR – International Commission for the Protection of the Danube River.
7.2 MZB -2 by Van Veen grab	Suitable for soft (sand, clay, silt) substrates; Can provide abundance / m^2.	Difficult / impossible to use on hard / clayey substrates; requires sampling plan and recording of location coordinates; at least three repetitions / station.	Written by REVIVO, Source: 2018: Van Veen grabs Manual, Eijkelkamp Soil & Water. 4p.
7.3 MZB – 3 by towed dredge	Alternative method for soft substrates; useful in stations of low MZB abundance.	Requires accurate recording of towing distance; difficult to use in high current conditions.	Written by REVIVO, Source: Graf W., Csányi B., Leitner P., Paunović M., Huber T., Szekeres J., Nagy C., Borza P. (2014). Full report on: Macroinvertebrates, Joint Danube Survey 3. ICPDR – International Commission for the Protection of the

			Danube River.
8. **Using cartographic materials to identify potential key-habitats**			
8.1 Navigation maps	Easy to access on internet; provide water depth in the channel; best to be combined with Google Earth.	Difficult to get coordinates and measure length and width of potential sites of interest.	Written by DDNI, Source: https://www.afdj.ro/en/content/inland-enc
8.2 Google Earth	Easy to access on internet; easy to measure width and length of potential reaches of interest, easy to obtain and record coordinates; easy to mark places of interest; easy to spot rocky banks; best to be combined with navigation maps.	No data of water depth.	Written by DDNI, Source: https://earth.google.com/web/
9. **Mapping riverbed morphology**			
9.1 Transects recorded with handheld sonar and GPS	The cheapest method; if conducted on different directions it can detect holes, sills and stairs.	Requires use of boat and at least 2 staff members; Records only the general shape of the river bottom.	Written by DDNI, Source: Hont S. et al. 2018, Review & Completion of the Feasibility Study for the Improvement of Navigation along the Joint Danube Romanian - Bulgarian Sector & Complementary Studies “FASTDANUBE”, Consultancy

			Contract 965 HRO
9.2Transects recorded with fish finder sonar	Easy to record transects and detect holes, sills and stairs; distinguishes between hard and soft substrate; records depth of soft substrate. Newer sonars can produce 3D bathymetry maps of transects.	Requires use of boat and at least 2 staff members.	Written by REVIVO, Source: Kaeser A.D., Litts T.L., Wesley T., T. (2012). Using Low-Cost Side-Scan Sonar For Benthic Mapping Throughout The Lower Flint River, Georgia, USA. River Research and Applications.
9.3Multi-beam sonar	Ideal tool to map important wintering, spawning, and nursery habitats; maps can be investigated using Global Mapper software.	Very expensive; requires expensive software to be acquired separately; requires using specialized boat and at least 2 operator personnel.	Written by DDNI, Source: Cochrane et al. (2003). Quantification of multibeam sonar, J. Acoust. Soc. Am., Vol. 114, No. 2, August 2003
10.**Capturing adults**			
10.1 Trammel nets	Effective when river bed morphology allows for bottom drifting of trammel nets.	Cannot be used on river beds with rough (rocky) bottom substrate.	Written by DDNI, Source: Hubert, W., A., Schmitt, D. N. (1982). Factors Influencing Catches of Drifted Trammel Nets in a Pool of the Upper Mississippi River," Proceedings of the Iowa Academy of Science, 89(4), 153-154. Available at: https://scholarworks.uni.edu/pias/vol89/iss4/5
10.2 Gill nets	Effective when installed on the "shoulder" of the channel preferred by sturgeons or other fish moving u/s.	Require timely recovery of nets to avoid suffocation of fish.	Written by DDNI, Source: Gessner J., Arndt, G.M. (2007). Modification of gill nets to minimize by catch of sturgeons, Journal of Applied Ichthyology 22(s1):166 – 171, DOI: 10.1111/j.1439-0426.2007.00946.x

10.3 Electro fishing	Effective in lower depth reaches of the river.	Cannot be used for capturing adult sturgeons.	Written by DDNI, Source: Moser, M. L., Conway J., Thrope T. (2000), Effects of recreational electrofishing on sturgeon habitat in the Cape Fear River drainage, Center for Marine Science Research, Final Report to North Carolina Sea Grant, Fishery Resource Grant Program
10.4 Baited hook lines	Effective in capturing bottom feeding species, including sterlets.	Require adapting hook size and bait to the species targeted; can harm / kill fish swallowing the hook; use to be avoided in case of critically endangered species such as sturgeons.	Written by DDNI, Source: Paighambari S., Y., Eighani M. (2017), Study on different hook and bait types in the Persian Gulf hand line fishery: optimization and development, Aquatic Living Resources 30:23 DOI: 10.1051/alr/2017007
11. **Using electro-narcosis**			
11.1 Handling during fishing (SEN-01)	Reduces catch induced stress in large size sturgeons considerably; eases handling and transport of very large fish to the bank.	Due to resemblance with electrofishing device in some countries it will require special permits to use on rivers.	Written by DDNI, Source: Suciu et al. (2014). Towards a healthy Danube fish migration iron gates I & II (Project Report), 20 October 2014 - 078017031:0.5 - Final C01011.200060.0100/SD
11.2 Tranquilizing for surgical implanting (electronarcosis tube)	Enables surgical implanting of tags without removal of the large sturgeon specimens from the water.	In some countries it will require animal welfare permits.	Written by DDNI, Source: Iani, M., Honţ, Ş., Paraschiv, M., Suciu R. (2017). A novel electro-narcosis method to anaesthetize adult sturgeon in the river during surgical implantation of acoustic tags. Scientific Annals of the Danube Delta Institute Vol. 22 Tulcea, Romania
11.3 Tranquilizing for handling during controlled propagation	Effective for tranquilizing large sturgeon males for sperm extraction	Cannot be used for striping eggs from sturgeon females because of the possibility of coming in contact with	Written by DDNI, Source: Hudson, J., Johnson, J., & Kynard, B. (2011). A Portable Electronarcosis System for Anesthetizing Salmonids and Other Fish. North American

(12 V DC electrodes in tank)	without removing the fish from the water tank.	water before fertilization.	Journal of Fisheries Management - NORTH AM J FISH MANAGE. 31. 335-339. 10.1080/02755947.2011.578524.
12.**Tracking movements using telemetry to demonstrate habitat use**			
12.1 Wintering sites in the river	Long battery life of large acoustic transmitters enables observation over at least 2 wintering events / seasons.	Requires mobile tracking during winter time with adequate boat and logistics.	Written by DDNI, Source: Xinhai, L., Matthew, K. L., Clarke J. E. H. (2007). Overwintering habitat use of shortnose sturgeon (Acipenser brevirostrum): defining critical habitat using a novel underwater video survey and modeling approach. Can. J. Fish. Aquat. Sci./J. can. sci. halieut. aquat. 64(9): 1248-1257
12.2 Wintering sites in the Black Sea	Satellite pop-up tags enable gathering data from adult sturgeons over very large marine areas, including successive locations during 1-2 years, swimming depth and water temperature preferences.	Results depend very much on chance to have recorded data transmitted to the Argos satellites, given the very high radio traffic over the space of the Black Sea.	Written by DDNI, Source: Suciu R., Paraschiv, M., Iani M., Honț S., Ionescu T. (2018). Habitats of sturgeons in the Lower Danube River and the North Western Black Sea: status of knowledge a[illegible] Conference on River habitat restoration for inland fisheries in the DRB and adjacent Black Sea areas. 13-15 November 2018, Bucharest, Romania
12.3 Spawning location and behavior	Effective when combining mobile tracking with submerged data-logging receivers.	To be effective it requires using / installing an array of submerged receivers to cover the whole migration range of the species in the river.	Written by DDNI, Source: Suciu R., Suciu M., Paraschiv M. (2005). Contributions to spawning ecology of beluga sturgeons (*Huso huso*) in the Lower Danube River, Romania. Extended Abstract of ISS 5 General Biology- Life History - CITES - Trade & Economy, Ramsar, Iran: 309 – 311
12.4 Feeding sites	Feeding behavior	Satellite telemetry	Written by DDNI,

in the Black Sea	(locations, itineraries, swimming depth and water temperature preferences) of adult sturgeons in the Black Sea can be reconstituted from data downloaded from pop-up tags via satellites.	equipment is very expensive, this being a factor limits its use on a large enough number of individuals to obtain statistically significant data.	Source: Holostenco D., Onără D. F., Suciu R., Honț Ș., Paraschiv M. 2013 Distribution and genetic diversity of sturgeons feeding in the marine area of the Danube Delta Biosphere Reserve. IN: Scientific Annals of the Danube Delta Institute, 19: 25 - 34, DOI: 10.7427/DDI.19.04 Maximov V. et al. 2014 Preliminary Data on the Monitoring of Sturgeon Species in Romanian Marine Waters
13.**Capturing eggs and larvae of benthic fish (D-shaped drift net)**	Effective if emerging time of free embryos or larvae can be predicted and nets are installed on the drifting path of larvae; most effective for sturgeons when deployed over-night.	Requires preliminary testing of exposure time to avoid over-filling of nets with bottom-rolling organic matter; best used if operating with two nets set in parallel across the width of the river.	Written by DDNI, Source: Report DDNI (2014). Assessment of Danube Delta Biosphere Reserve in relation to environme the project no. PN 09 no. 26N / ANCS / 2009, Additional Act no.1 2014 (executor: INCDDD - Tulcea). Tulcea, Romania.
14.**Capturing pelagic eggs and larvae of shads (*Alosa sp.*)**	Cheap and easy estimation of the relative abundance of juvenile shad using "filtered water volume".	The position of the sampling stations must cover the main water current.	Written by DDNI, Source: Năstase A., Năvodaru I., Cernișencu I., Țiganov G., Popa L.2018 Pontic shad (*Alosa immaculata*) migrating upstream the Danube river and larval drift downstream to the Black Sea in 2016. Scientific Annals of the Danube Delta Institute, Romania, vol. 23https://doi.org/10.7427/DDI.23.08
15.**Capturing YOY sturgeons**	Using 20 mm mesh of the inner net in bottom drifting trammel nets enables capturing a wide range of YOY sizes TL = 8 – 30 cm.	Requires predicting the start of YOY arrival at the fishing site.	Written by DDNI, Source: Eugenia C. (2019). Research on the state of ecologically reconstructed areas, anthropically degraded areas and recovery of sturgeon species in RBDD. 24 pages. Report Phase 4 / December / 2019, of the project no. PN 19 12 02 02 04 (coord. Dr eng. Eugenia CIOACĂ) of the contract no. 41N / 2019 / MCI, executor: INCDDD - Tulcea. Tulcea, Romania.
16.**Monitoring**	Cheapest and most	Requires conducting	Written by DDNI,

annual recruitment of sturgeons	effective way of assessing the status of sturgeon populations.	monitoring for many years to understand the status and trend of populations.	Source: Report DDNI (2018). Studies on the state (hydrology, vegetation, substrate, hydro-morphological changes) of the sturgeon breeding and feeding areas in the C Phase III / December / 2018, of the project no. PN 18 14 02 02 (coord. Jenică HANGANU) of the contract no. 14N / MCI / 2018, (executor: INCDDD - Tulcea). Tulcea, Romania.
16. **Sampling YOY**			
16. 1 Keeping and handling in aerated holding tanks	Mobile, battery operated aerators in 20 L holding tanks are useful to keep YOY for 1-2 hours while lifting the net and transporting to the camp for measurements, photos and sampling.	However, water should be exchanged several times; sterlet YOY cannot be mixed with other species because they produce and eliminate excessive mucus in the holding tank (which affects other species).	Written by DDNI, Source: Paraschiv M., Suciu, R., Onără D, Holostenco D., Iani M., Taflan L. (2011) Cercetări privind reproducerea şi dezvoltarea sturionilor în Dunăre. FAZA III/ 2011: Cercetări privind hrănirea puilor de sturioni în Dunăre în anul 2011 COD PROIECT: PN 09 26 05 07,Beneficiar: Ministerul Educaţiei, Cercetării, Tineretului şi Sportului, Autoritatea Naţională pentru Cercetare Ştiinţifică, Executant: Institutul Naţional de Cercetare-Dezvoltare Delta Dunării
16.2 Stomach content (gastric lavation)	Should be performed first to take advantage of still lively YOY sturgeons; Most efficient on sterlet YOY.	Difficult procedure on YOY Beluga sturgeons (which at the size of TL 8 cm already feed on larvae of other fish species).	Written by DDNI, Source: Baker, R., Buckland, A. and Sheaves, M. (2014). Fish gut content analysis: robust measures of diet composition. Fish Fish., 15 (1): 170-177. Buckland, A., Baker, R., Loneragan, N., Sheaves, M. (2017). Standardising fish stomach content analysis: the importance of prey condition. Fisheries Research, 196. pp. 126-140.
16.3 Biometric characteristics & photos	YOY sturgeons can be measured and weighted and as their body movements slow down photos can be taken by aligning the fish to a	Avoid exposing the fish to full sunshine.	Written by DDNI, Source: Onără D. (2015). Research to argue the sustainable exploitation of sturgeons that breed in the lower Danube, in order to establish harmless catch quotas, with a view to the reopening of commercial sturgeon fishing in 2016 (project PN 09-26 05 09), (Coord. Dr. Dalia Onără) CONTRACT

	measuring tape and a label with the site, date and the sample code of the fish.		Nr. 26N / 2009 cod PN 09-26 Phase I
16.4 Mark and recapture (FFT tags)	FFT tags can be attached easily and quickly.	Fish should be already displaying slow movements to avoid interfering with the tag attachment procedure.	Written by DDNI, Source: Thorsteinsson, V. et research in fisheries. Report of Concerted Action FAIR CT.96.1394 (CATAG), Marine Research Institute, Skulagata 4, IS-121 Reykjavik, Iceland
16.5 Tissue samples	Are taken as the last operation before placing the fish in the holding tank.	Clipping the anal fin is causing the least impact on the normal swimming activity of YOY sturgeons.	Written by DDNI, Source: Onără, D., Holoștenco, D., Paraschiv, M., Suciu, R. (2014). Preliminary genetic variability of Lower Danube River young of the year (YOY) beluga sturgeon *Huso huso* (Linnaeus, 1758) using mtDNA markers. Journal of Applied Ichthyology 30: 1286-1289.
17. **Tracking movement and nursery habitat use by YOY (acoustic telemetry)**	Small acoustic transmitters can be implanted to YOY sturgeons of TL ≥ 18 - 20 cm using chemical anesthetics, cooling of water and continuous monitoring of oxygen during each sequence of the procedure.	Not advisable / possible to use in wild YOY sturgeons unless they are kept for advanced healing of the wound; otherwise YOY will drift with the river current and not provide any behavioral information.	Written by DDNI, Source: Cristea V. et al. (2016). Assessment of the survival and spread in the Black Sea of sturgeon chicks of critically endangered species, launched in the Lower Danube Romania (2013 – 2015); Available at:: http://www.ddni.ro/sturgeons/images/Files/rapstprpilot2013.pdf (in romanian)

Table 2 shows the list of equipment with the costs required to implement each research method. Organizations and/or individuals can use provided data for easier financial planning of a similar activities.Each method also requires use of inflatable or hard bottom boat and outboard boat engine 15-20 HP. As some institutions already own a boat and an outboard engine, these costs are not included under equipment cost of each method. Therefore, if necessary, include purchase or rental of a boat and outboard engine (new boat – app. € 5,500, new outboard engine 15-20HP – app. €3,500; maintenance costs per year – €600or boat rental including outboard engine – €100 per day).

Table 4 : Estimated costs and personnel for migratory fish habitat assessment methods.

Method	**Equipment**	**Approx. cost [€]**	**Annual operational costs [€]**	**Personnel and level of experience**	**Source**
1. **Substrate identification**					
1.1 Kynard probe	Kynard probe, small boat with 15-20 HP engine, handheld sonar, GPS, Van Veen grab, camera, work equipment on the river.	Kynard probe – € 50 or less (if self-made) Hand held sonar – € 250 GPS – € 350 Van Veen grab – € 1,500 Camera – € 170 Other working equipment – € 100	€200	2 people with experience in using the equipment and working on the river	Source: REVIVO, 2021
1.2 Side scan sonar	Complete RiverSurveyor M9 system, (boat with 15-20 HP engine, work equipment on the river).	RiverSurveyorM9 system – € 50,000 Other working equipment – € 100	€500	2 people with experience in using the equipment and working on the river	Source: DDNI, 2021
1.3 Low budget Fishfinder sonar	Hummingbird Helix 7 CHIRP SI GPS G2N system with the XNT 9 SI 180 T probe or similar, (boat with 15-20 HP engine, work equipment on the river).	Fishfinder sonar – € 1,500 Car battery – € 50 Car battery charger – € 50 Other working equipment – € 100	€200	2 – 3 people with experience in using the equipment and working on the river	Source: REVIVO, 2021
1.4 Van Veen grab	Van Veen grab, (boat with 15-20 HP engine, work equipment	Van Veen grab – € 1,500 Other working equipment – € 200	€100	3 people with experience in using	Source: REVIVO,

	on the river).	GPS – € 350		the equipment and working on the river	2021
1.5. Water Wolf camera	WaterWolf UW 1.1. camera, (boat with 15-20 HP engine, work equipment on the river)	Water Wolf Camera – € 170 Long rope with weight – € 40 (or long stick) GPS – € 350 Other working equipment – € 100	€200	2 people with experience in using the equipment and working on the river	Source: REVIVO, 2021
2. **Water velocity**					
2.1 Electro-magnetic flowmeter	Electromagnetic flowmeter[1], Hydrological winch and USGS Columbus-Type Sounding Weight, Epoxy Coated, 15lb[2], support for fixing the winch on the boat (boat with 15-20 HP engine, work equipment on the river).	Flowmeter rental per month – € 800 Hydrological winch – € 8,000 Mouth system for winch installation – € 500 Sounding Weight – € 400 GPS – € 350 Other working equipment – € 200	€500	3 people with experience in using the equipment and working on the river	Source: DDNI, 2021
2.2 ADCP	ADCP[3], (boat with 15-20 HP engine, work equipment on the river).	ADCP rental per month – € 3,200 GPS – € 350 Other working equipment – € 100	€200	experience in using the equipment and working on the river	DDNI, 2021
3. **Discharge dynamics**					
3.1 Water level records by navigation authority	1 PC with internet connection.	Computer with internet connection – € 1,000	€500	a person with experience in working on the internet and databases	Source: REVIVO, 2021
3.2 Discharge recorded by regional water	(1 PC with internet connection).	See row 3.1.	See row 3.1.	a person with experience in working on the	Source: REVIVO, 2021

management authorities				internet and databases	
3.3 ADCP	(ADCP, boat with 15-20 HP engine).	See row 2.2.	See row 2.2.	3 people with experience in using the equipment and working on the river	Source: DDNI, 2021
4. **Water depth**					
4.1 Hand-held sonar	Hand-held sonar, (boat with 15-20 HP engine, work equipment on the river).	Hand held sonar – € 250 Other working equipment – € 50	€100	2 people with experience in using the equipment and working on the river	Source: REVIVO, 2021
4.2 Fishfinder sonar	Fishfinder sonar, car battery, (boat with 15-20 HP engine, work equipment on the river).	Fishfinder sonar – from €200 up to €1,500 Car battery – € 50 Car battery charger – € 50 Other working equipment – € 50	€200	2 people with experience in using [illegible]	Source: REVIVO [illegible]
5. **Water temperature**					
5.1 Multi-meter	Multi-meter, (boat with 15-20 HP engine, work equipment on the river).	Multi-meter – € 2,000 GPS – € 350 Other working equipment – € 50	€200	2 people with experience in using the equipment and working on the river	Source: DDNI, 2021
5.2 Submerged temperature recorder	Data logger, (boat with 15-20 HP engine, work equipment on the river).	Temperature data logger – € 400 GPS – € 350 Other working equipment – € 50	€200	2 people with experience in using the equipment and working on the river	Source: DDNI, 2021
6. **Turbidity**					
6.1 Secchi Disk Visibility (SDV)	Secchi Disk, (boat with 15-20 HP engine, work equipment on the river).	Secchi Disk – € 100 GPS – € 350 Other working equipment – € 50	€100	2 people with experience in using the equipment and	Source: DDNI, 2021

				working on the river	
6.2 Turbidimeter (data logging)	Turbidity logger and accessories[4], (boat with 15-20 HP engine, work equipment on the river).	Turbidity logger and accessories – € 2,900 GPS – € 350 Other working equipment – € 50	€500	2 people with experience in using the equipment and working on the river	Source: DDNI, 2021
7. **MZB abundance and composition**					
7.1 MZB -1 by stationary drift net	Stationary drift net and accessories, (boat with 15-20 HP engine, work equipment on the river, hand held sonar, GPS, camera).	Stationary drift net and accessories – € 2,500 GPS – € 350 Hand held sonar – € 250 Camera – € 170 Other working equipment – € 50	€1,000	2 people with experience in using the equipment and working on the river	Source: DDNI, 2021
7.2 MZB -2 by Van Veen grab	Van Veen grab, rope, filter, (boat with 15-20 HP engine, work equipment on the river, hand held sonar, GPS, camera)	Van Veen grab – € 1.500 Filter – € 100 Rope – € 20 GPS – € 350 Hand held sonar – € 250 Camera – € 170 Other working equipment – € 200	€300	2 people with experience in using the equipment and working on the river	Source: DDNI, 2021
7.3 MZB – € 3 by towed dredge	Towed dredge, rope, (boat with 20 HP engine, work equipment on the river, hand held sonar, GPS, camera)	Towed dredge – € 2,500 Rope – € 20 GPS – € 350 Hand held sonar – € 250 Camera – € 170 Other working equipment – € 200	€1,000	2 -3 people with experience in using the equipment and working on the river	Source: DDNI, 2021
8. **Using cartographic materials to**					

identify potential key-habitats					
8.1 Navigation maps	Navigation maps.	Navigation maps – € 100 Computer with internet connection – s row 3.1.		A person familiar maps	Source: 2021
8.2 Google Earth	(1 PC with internet connection).	Computer with internet connection – see row 8.1.	See row 8.1	a person with experience in working on the internet	Source: REVIVO, 2021
9. **Mapping riverbed morphology**					
9.1 Transects recorded with handheld sonar and GPS	(Boat with 15-20 HP engine, work equipment on the river, hand held sonar, GPS).	Hand held sonar – € 250 GPS – € 350 Other working equipment – € 200	€100	2 people, at least one of whom should know this procedure	Source: DDNI, 2021
9.2 Transects recorded with fishfinder sonar	(Fishfinder sonar with GPS sensor, car battery, (boat with 15-20 HP engine, work equipment on the river).	Fishfinder sonar – from €350 up to €1,500 Car battery – € 50 Car battery charger – € 50 Other working equipment – € 100	€300	2 people with experience in using the equipment and working on the river	Source: REVIVO, 2021
9.3 Multi-beam sonar	Multi-beam sonar, (boat with 15-20 HP engine, work equipment on the river).	Multi-beam Sonar –€60,000 Car battery – € 50 (if necessary) Car battery charger – € 50 (if necessary) Other working equipment – € 100	€1,000	2 people with experience in using the equipment and working on the river	Source: DDNI, 2021
10. **Capturing adults**					
10.1 Trammel nets	3 x 100m trammel nets, buoys, ropes, anchors, (boat with 15-20 HP engine, work equipment	3 X 100 m trammel nets –€1,800 6 X buoys – € 420 120 m ropes – € 100	€1,500	3 people with experience in using the equipment and	Source: REVIVO, 2021

	on the river, hand held sonar, GPS), etc.	6 X anchors – € 180 GPS – € 350 Hand held sonar or Fishfinder sonar – from €250 up to €1,500 Other working equipment – € 100		working on the river	
10.2 Gill nets	Gill nets, (buoys, ropes, anchors, boat with 15-20 HP engine, work equipment on the river, hand held sonar, GPS).	2 X 100 m gill nets – € 600 2 X buoys – € 140 120 m ropes – € 100 4 X anchors – € 120 GPS – € 350 Hand held sonar or Fishfinder sonar – from €250 up to €1,500 Other working equipment – € 100	€1,500	2 people with experience in using the equipment and working on the river	Source: REVIVO, 2021
10.3 Electro fishing	Electrofishing unit, (Secchi Disk, Multi-meter, boat with 15-20 HP engine, work equipment on the river, hand held sonar, GPS).	Electrofishing unit – € from 4,500 up to 12,000 Secchi Disk – € 100 Multi-meter – € 2,000 Hand held sonar or Fishfinder sonar – from €250 up to €1,500 GPS – € 350 Other working equipment (life jackets, polaroid glasses, fishing waders, buckets etc.) – € 1,500	€2,500	2 people with experience in using the equipment and working on the river	Source: DDNI and REVIVO, 2021
10.4 Baited hook lines	Baited hook lines, (boat with 15-20 HP engine, work equipment on the river, hand held sonar, GPS).	1 X baited hook lines – € 100 GPS – € 350 Hand held sonar or Fishfinder sonar – from €250 up to€ 1,500 Other working equipment – € 100	€1,000	2 people with experience in using the equipment and working on the river	Source: REVIVO, 2021
11. **Using electro-narcosis**					
11.1 Handling	Electronarcosis unit, special	Electrofishing unit – from €4,500 up to	€2,000	2 fishermen who	Source:

during fishing (SEN-01)	electrodes for boat, car battery (boat with 15-20 HP engine, work equipment on the river, hand held sonar, GPS).	€12,000 Hand held sonar or Fishfinder sonar – from €250 up to€ 1,500 GPS – € 350 Car battery – € 50 Car battery charger – € 50 Other working equipment (life jackets, polaroid glasses, fishing waders, buckets etc.) – € 1,500		have been trained in the use of the electronarcosis gear	DDNI and REVIVO, 2021
11.2 Tranquilizing for surgical implanting	Electronarcosis tube**,** (Electronarcosis unit, car battery, boat with 15-20 HP engine, work equipment on the river, etc.).	Electronarcosis tube –€600 Hand held sonar or Fishfinder sonar – from €250 up to €1,500 GPS – € 350 Car battery – € 50 Car battery charger – € 50 Other working equipment (life jackets, polaroid glasses, fishing waders, buckets etc.) – € 1,500	€500	2 people with experience in using the equipment, and surgical implanting	Source: DDNI, 2021
11.3 Tranquilizing for handling during controlled propagation	Electronarcosis basin, stretcher for transporting fish (Electronarcosis unit, car battery. etc.).	Electronarcosis basin –€600 Car battery – € 50 Car battery charger – € 50 Other working equipment– €500	€300	2 – € 3 people with experience in using the equipment	Source: DDNI, 2021
12. **Capturing eggs and larvae of benthic fish** (D-shaped drift net)	(D-shaped drift net, boat with 15-20 HP engine, work equipment on the river, GPS, fish finder sonar, etc.).	2 X D – € shaped drift net – € 3,000 4 X buoys – € 280 120 m ropes – € 100 4 X anchors – € 120 GPS – € 350 Hand held sonar or Fishfinder sonar – from €250 up to €1,500 Other working equipment – € 100	€1,000	2 people with experience in using the equipment and working on the river	Source: DDNI and REVIVO, 2021
13. **Capturing**	Bongo net, (boat with 15-20	1 X bongo net – € 1,000	€1,500	2 people with	Source:

pelagic eggs and larvae of shads (*Alosa sp.*)	HP engine, work equipment on the river).	30 m ropes – € 15 GPS – € 350 Hand held sonar or Fishfinder sonar – from €250 up to €1,500 Other working equipment – € 100		experience in using the equipment and working on the river	DDNI, 2021
14. **Capturing YOY sturgeons - Monitoring annual recruitment of sturgeons**	Gill nets, (buoys, ropes, boat with 15-20 HP engine, work equipment on the river, hand held sonar, GPS, camera, etc,).	2 X 100 m gill nets – € 600 2 X buoys – € 140 60 m ropes – € 30 GPS – € 350 Camera – € 170 Hand held sonar or Fishfinder sonar – from €250 up to €1,500 Other working equipment – € 100	€2,000	2 people with experience in fishing YOY on the river	Source: DDNI, 2021
15. **Sampling YOY**					
15. 1 Keeping and handling in aerated holding tanks	Holding tanks, aerators, batteries.	Holding tank –€20 Aerator – € 50 Batteries – € 20 Other working equipment – € 50	€300	One person	Source: DDNI, 2021
15.2 Stomach content (gastric lavation)	Device for collecting stomach contents, airtight containers for preserving the stomach content in alcohol.	Device for collecting stomach contents – € 50 50 X containers for preserving the stomach content in alcohol – € 10 Other working equipment – € 50	€300	2 persons	Source: DDNI, 2021
15.3 Biometric characteristics & photos	Measuring ruler, scale (camera).	Measuring ruler – € 10 Scale – € 15 Camera – € 170 Other working equipment – € 50	€200	2 persons	Source: DDNI, 2021
15.4 Mark and recapture (FFI	FFI tags.	FFI tags – € 300 Other working equipment – € 50	€150	2 persons	Source: DDNI,

tags)					2021
15.5 Tissue samples	Containers with absolute ethyl alcohol for preserving samples, scissors etc.	Containers with absolute ethyl alcohol – € 150 Other working equipment – € 50	€150	2 persons	Source: DDNI, 2021
16. **Acoustic telemetry - tracking movement of fish between wintering, spawning, feeding habitats and nursery habitat used by YOY**	e.g. 50 acoustic tags, 10 receivers VR2w, 1 receiver VR100, 10 buoys, 10 anchors, 300 m steel cable, 1 field laptop, etc.	50 acoustic tags – € 15,000 10 X receivers VR2w – € 12,000 1 receiver VR100 – € 5,000 10 X buoys – € 2,000 10 X anchors – € 500 300 m steel cable – € 300 1 field computer – € 1,000 GPS – € 350 Other working equipment – € 300	€3,500	3-4 persons	Source: DDNI, 2021

[1] *https://www.fieldenvironmental.com/equipment-rentals/water-monitoring-environmental-equipment/water-measurement/flow-probes-and-meters/in-stream/marsh-mcbirney-flo-mate.html;*
[2] *https://rickly.com/usgs-columbus-type-sounding-weight-epoxy-coated-15lb;*
[3] *https://www.fondriest.com/hydrosurveyor-adcp-rental.htm;*
[4] *https://store.eonpro.com/store/p/2286-Aquistar-Turbidity-Logger.aspx;*
[5] *In shallower and narrower rivers can be replaced by radio telemetry;*