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NINA Report

Restoration of fish migration in the Danube River at Iron Gate Dams in Romania and Serbia

Studies of fish behaviour in 2019 and 2021

Marian Paraschiv, Finn Økland, Mirjana Lenhardt, Rachel A. Paterson, Stefan Hont, Gorcin Cvijanovic, Torgeir B. Havn, Marian Iani, Marija Smederevac-Lalic, Miroslav Nikčević, Nicușor Neacșu, Dušan Nikolić and Eva B. Thorstad



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Iron Gate Dam II, Danube © Eva B. Thorstad

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- tagging, electronic tag, acoustic transmitter, telemetry

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Abstract

Paraschiv, M., Økland, F., Lenhardt, M., Paterson, R.A., Hont, S., Cvijanovic, G., Havn, T.B., Iani, M., Smederevac-Lalic, M., Nikčević, M., Neacșu, N., Nikolić, D. & Thorstad, E.B. 2021. Restoration of fish migration in the Danube River at Iron Gate Dams in Romania and Serbia. Studies of fish behaviour in 2019 and 2021. NINA Report 2030: 1-36.

The Iron Gate hydropower and navigation system is one of the largest river engineering projects in Europe, with the dams built to provide hydropower and facilitate navigation along the Danube. The Iron Gate Dam I was completed in 1972 and Iron Gate Dam II in 1984. The border between Romania and Serbia follows the Danube in this area, and the two countries share both dams.

Before the construction of the dams, fish were able to migrate from the Black Sea to the upper parts of the Danube and its tributaries. The dams blocked upstream fish migration, and no fish passes have been constructed. Constructing fish passes at the Iron Gate Dams would open up 960 km of the Danube River to the Gabčíkovo Dam (Slovakia) to upstream migrating sturgeon and other fishes, and in addition access to numerous tributaries.

Well-functioning fish passes require that migrating fish are able to find and use the entrance, and thereafter move through the entire fish pass. The entrances need to be located in areas where migrating fish seek a passable route past the dam. To establish functional fish passes is particularly challenging at large dams where there is a long distance between the river banks and large areas where the fish can migrate and aggregate.

Results from a feasibility study of the behaviour of asp, barbel, common carp, common nase, Pontic shad and vimba bream in autumn 2019 and spring 2021 are presented in this report, as a step towards identifying suitable areas for fish pass entrances at the Iron Gate Dams. The specific aims were to examine behaviour and distribution of fish downstream of the Iron Gate Dam II and in the Gogosu branch, movements in the reservoir between the Iron Gate Dam I and Iron Gate Dam II, and behaviour and distribution of fish downstream of the Iron Gate Dam I, if tagged fish would reach this far.

The study was performed by tagging fish with acoustic transmitters and releasing them downstream and upstream of Iron Gate Dam II. The movements of tagged fish were recorded by deploying receivers at strategic places in the river. Tagged fish were automatically recorded when they were within the detection range of the receivers.

A total of 185 fish were tagged; 61 in autumn 2019 and 124 in spring 2021. The most common species among the tagged fish were vimba bream and common nase, followed by barbel and asp.

Vimba bream, common nase, barbel and asp are known to be migratory species, which can perform long-distance movements. This was confirmed also for our study area, with many tagged individuals performing extensive movements, both below the Iron Gate II dam, in the Gogosu branch and in the reservoir. Several barbel, common nase and vimba bream moved upstream through the entire reservoir to the Iron Gate I dam, which was 76 km from the release site in the lower part of the reservoir. Barbel and vimba bream were the two species showing the most extensive movements. The study also confirmed that there is large individual variation in movement strategies and behaviour within these species.

The detailed behaviour of the fish when approaching the Iron Gate II and Iron Gate I dams was studied by deploying several receivers with different detection ranges in the areas below the dams. The fish showed great individual variation in where they approached the dams, and most of the individuals were recorded by receivers on both sides of the river. Hence, a preference for moving along one particular side of the river was not identified for any of the species. Studies of detailed behaviour of tagged fish can only be as fine-scaled as the receiver deployment allows.

In the present study, a relatively limited number of acoustic receivers was used, limiting the analysis of detailed behaviour below the dams. Further studies will be performed to increase the information of the detailed fish behaviour below the dams.

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Foreword

Results from studies of fish behaviour at the Iron Gate Dams on the Danube River are presented in this report. The data have been collected by tagging individual fish with acoustic transmitters and recording their subsequent movements. The report has been prepared as part of Task 3 of the WePass project, which is a project focused on facilitating fish migration and conservation at the Iron Gate Dams in the Danube River in Romania and Serbia.

The WePass project was funded by a grant awarded by the European Commission (DG REGIO) for the action *Study on environmental and ecological thematic in the framework of MRS and policy coordination with DG NEAR/ENV: Support for the Implementation of the Feasibility Study analysing options for fish migration at Iron Gate I & II* (agreement number 2018CE160AT019). The project has been coordinated by the International Commission for the Protection of the Danube River (ICPDR).

The studies described in this report were performed through a collaboration between scientists from the Danube Delta National Institute (Romania), the Institute for Multidisciplinary Research University Belgrade (Serbia) and the Norwegian Institute for Nature Research (Norway).

We would like to thank Edith Hödl, project manager of the WePass project at the ICPDR Secretariat, for support during the work. We would like to thank Igor Škodrić (Prahovo, Serbia) and Milan Prvanović (Zaječar, Serbia) for help with catching fish during the fieldwork, Kari Sivertsen (NINA, Norway) for help with graphic design of maps used in the report, and Jörn Gessner (IGB Berlin, Germany) for helpful comments to a previous version of the report.

Romania/Norway November 2021

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Introduction

Fish migration and river fragmentation

Like other animals, many fishes perform migrations during their lifetime. The best place to breed may not be the best place to find food, and spawning and feeding migrations may be a strategy to utilise the best areas during different life stages. Seasonal migrations may also be a strategy to avoid hostile conditions during parts of the year.

Rivers worldwide have become increasingly modified for navigation, hydropower and water regulation purposes; negatively impacting river habitat quality and connectivity, and as a result fish migration and survival. Dams built across rivers can block access to important feeding, spawning and nursery areas of fish if they are not equipped with suitable fish passes. Lost access to important habitats can lead to genetic depletion, severely reduced fish populations, and in the worst case, extinction.

Some fish species depend on migrations within river systems, whereas other species may depend on migrations between rivers and the sea. For all these species, safe and efficient fish passes are needed to support up- and downstream migrations where the connectivity has been lost.

Iron Gate Dams

The Iron Gate hydropower and navigation system is one of the largest river engineering projects in Europe, with the dams built to provide hydropower and facilitate navigation along the Danube. The Iron Gate Dam I was completed in 1972 and Iron Gate Dam II in 1984. The border between Romania and Serbia follows the Danube in this area, and the two countries share the dams (figure 1).

Before the construction of the dams, fish could migrate from the Black Sea to the upper parts of the Danube and its tributaries. The dams blocked upstream fish migration, and no fish passes have been constructed. The mortality rate of fish passing downstream through the hydropower turbines is unknown.



Figure 1. Map of the Danube and Iron Gate I and II dams at the border between Romania and Serbia.

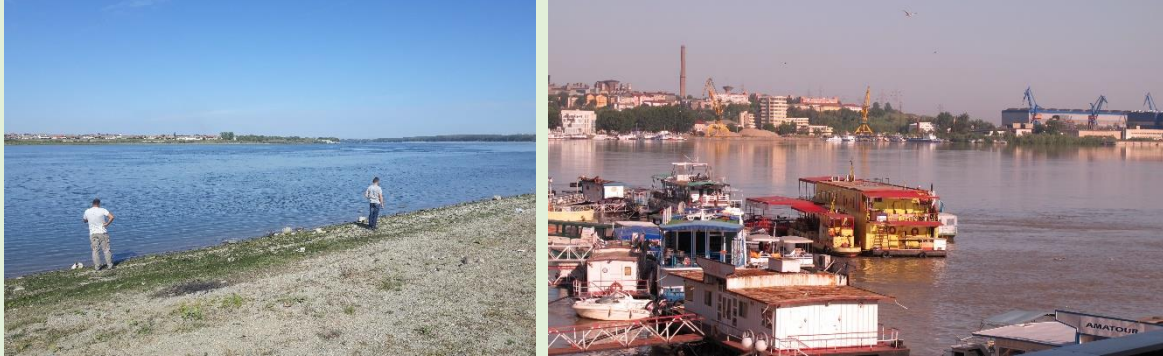
Fish migration routes can be re-established by building fish passes

The most famous fishes of the Danube are the sturgeons that spawn in the river and perform feeding migrations to the Black Sea. Sturgeon are among the most ancient fish families in the world, with most sturgeon species facing the risk of extinction. Before the construction of the Iron Gate Dams, sturgeons migrated from the Black Sea as far upstream as Austria and Germany. With the exception of the sterlet *Acipenser ruthenus*, a freshwater sturgeon species, the remaining three species of migratory sturgeons in the Danube (beluga *Huso huso*, Russian sturgeon *A. güldenstädtii*, and stellate sturgeon *A. stellatus*) are confined to the Lower Danube between the Iron Gate and the Black Sea as a result of the dam constructions. In addition to the sturgeons, there are numerous other migratory fishes that need to migrate up and down the Danube in order to fulfil their life cycles - and that would benefit from additional habitat and connection between the populations upstream and downstream of the dam - such as common nase *Chondrostoma nasus*, pikeperch *Sander lucioperca*, barbel *Barbus barbus*, vimba bream *Vimba vimba*, and asp *Leuciscus aspius*.

Constructing fish passes at the Iron Gate Dams would open up 960 km of the Danube River to the Gabčíkovo Dam (Slovakia) to upstream migrating sturgeon and other fishes, and in addition access to numerous tributaries. For sturgeon, this would include access to numerous spawning grounds along the Danube and major tributaries. Opening the migration routes for fish through the Iron Gate Dams would help to meet EU goals, including increasing the ecological status of rivers within the EU, and protecting biodiversity and threatened species. Re-establishing the connectivity for migratory fish species, moving both upriver and downriver in the Danube is top priority in the European Union (EU) due to the provisions of the Water Framework Directive and the implementation of the EU Strategy for the Danube Region. The EU Water Framework Directive provides legal requirements for the restoration of fish migration routes at national and international level to achieve the environmental objectives for European waters.

The Danube

The Danube is the second largest river in Europe, 2 850 km long, located in Central and Eastern Europe. It originates in Germany, flows through ten countries and drains into the Black Sea. The Danube Delta mainly lies in Romania and partially in Ukraine.



The Danube at the Romanian-Serbian border (left), and at Tulcea in Romania (right). Photos: Stefan Hont and Eva B. Thorstad.

Approximately 150 fish species occur in the Danube, such as asp, barbel, pike, common nase, huchen, zander, vimba bream, Wels catfish, Pontic shad, burbot, tench, European eel and sturgeons. Some of the species are fairly stationary freshwater fishes, some are migrating within the river system, and some migrate between the Danube and the Black Sea.



Stellate sturgeon in an aquarium in Tulcea. Photo: Eva B. Thorstad.

The Danube has been a traditional trade route since ancient times. The river is navigable by ships the entire stretch between Germany and the Black Sea. Photo: Eva B. Thorstad, at Tulcea in Romania.



Construction of well-functioning fish passes requires knowledge of fish behaviour

Well-functioning fish passes require that migrating fish are able to find and use the entrance, and thereafter move through the entire fish pass. Establishing functional fish passes is particularly challenging at large dams where distances between the shores are long, and there are vast areas where the fish can potentially migrate and aggregate. The entrances need to be located in areas where migrating fish seek a passable route past the dam.

Upstream migrating fish often follow the main water flow, but may select water velocities that reduce the energy required for upstream movement and avoid unfavourable hydraulic conditions like areas with too high water velocity and substantial turbulence. Information about the movements and distribution of fish trying to pass the dam is therefore a prerequisite to ensure that the fish pass entrances will be located in areas where they can be detected by as many upstream moving fish as possible.

Rivers are dynamic systems with flow varying both seasonally and annually. It is important that the planning, design and construction of passages are based on biological insight into how the fish behave at the particular dams, and how variable flow regimes and turbine loads influence the hydraulic conditions and distribution of fish below the dams.

Aims of study

Results from a feasibility study of the behaviour of asp, barbel, common carp *Cyprinus carpio*, common nase, Pontic shad *Alosa immaculata*, and vimba bream in autumn 2019 and spring 2021 are presented in this report, as a step towards identifying suitable areas for fish passes at the Iron Gate Dams.

The specific aims of the study were to examine:

- behaviour and distribution of fish downstream of the Iron Gate Dam II and in the Gogosu branch,
- movements in the reservoir between the Iron Gate Dam I and Iron Gate Dam II, and
- behaviour and distribution of fish downstream of the Iron Gate Dam I, if tagged fish would reach this far.

The study was performed by tagging fish with acoustic transmitters and releasing them downstream and upstream of Iron Gate Dam II. The movements of tagged fish were recorded by deploying receivers at strategic places in the river, where the fish were automatically recorded when they passed within the detection range of the receivers. Tagged fish were also recorded by manual tracking from boat by using a portable receiver.

The Iron Gates

The Iron Gates is a river stretch and a gorge along the Danube, forming the border between Romania and Serbia. Riverbed rocks and rapids made the gorge valley a difficult stretch to pass by boats. Since the 19th century, efforts have been made to allow passage of larger vessels.

In 1964, a Romanian-Yugoslavian project commenced the construction of the Iron Gate I and Iron Gate II dams, which were opened in 1972 and 1984 with hydropower stations, sluices and navigation locks for shipping. The construction of the dams created large reservoirs and increased the water level and water covered areas, and has had major impact on flora and fauna. The dams are obstacles to fish migration and have blocked access for the large sturgeon species to upstream spawning areas. Fish can pass the turbines in a downstream direction, but may die from injuries, and mortality rates are not known.

Iron Gate I

Iron Gate I is the largest dam (height 60 m, length 1278 m) on the Danube and one of the largest hydropower stations in Europe (annual generation of about 5.9 TWh, 12 Kaplan turbines). The hydropower station is divided in two sections, owned by Serbia on one side and Romania on the other. The reservoir upstream of the dam, the Iron Gate I reservoir, has a capacity of 2.1 km³ and a surface area of 104 km².



Iron Gate I. Photo: Eva B. Thorstad.



The Danube from Iron Gate I and looking downstream. Entrance to the ship lock on the Romanian side is seen in the left part of the photo. Photo: Eva B. Thorstad.

Iron Gate II

Iron Gate II (height 35 m, length 412 m) is situated 80 km downstream of Iron Gate I. Also at this site, the hydropower station is divided in two sections, owned by Romania and Serbia (annual generation of about 591 MW, 18 turbine units). The 80 km long reservoir upstream of the dam, the Iron Gate II reservoir, has a surface area of 104 km². Cars and light cargo vehicles can cross the river on the dam, and there is a border checkpoint between Romania and Serbia.



Iron Gate II. Photo: Finn Økland.

Gogosu branch

The Gogosu is a 15 km long side arm of the Danube on the Romanian side, which branches off the main river upstream of Iron Gate II and rejoins downstream of the dam. There is a dam with a hydropower station across the upper part of the Gogosu branch. The Romanian ship lock at the Iron Gate II area is constructed between the lower part of the Gogosu branch and the main river, and it connects to the main river about 2.5 km upstream of the dam.



The Gogosu branch from the dam and looking downstream. Photo: Finn Økland.



Location of Gogosu branch re-entry to the main channel of the Danube downstream of Iron Gate II. Arrow to the left shows Iron Gate II and arrow to the right the lower end of the Gogosu branch. Photo: Eva B. Thorstad.

Methods

Acoustic telemetry

Fish movements and migrations can be tracked using acoustic telemetry methods. The fish is tagged with a transmitter, also called tag, that emits acoustic sound signals (called “ping”). The tag is usually implanted into the body cavity of the fish.

The tag signals are detected when the fish is near a receiver with an acoustic antenna, called a hydrophone. Signals can only be detected in water, so receivers must be placed in water.

Automatic stationary receivers can be deployed at different sites in the study area. The receivers store data on time and tag ID, which is unique for each tagged fish, every time it detects a signal. Transmitters are programmed to emit signals typically every few seconds or minutes.

When a tagged fish is not close enough to a receiver for the signals to be detected, it is not known where the fish is. However, it is possible to search for tagged fish in the study area by using a portable receiver that is moved around, usually by using a boat.

How far from the fish can we detect signals from a tag?

The detection range is largely determined by the distance the signal from the tag can travel through water, which again is much dependent on the strength of the signal produced by the tag. Stronger signals require more battery power. Since batteries must be small to keep the tags small enough for the fish, the range is limited. Features of receivers also impact the range.

The range is typically a few hundred meters. However, the range is strongly affected by environmental conditions in the water, and for instance turbulent water containing oxygen bubbles can reduce the range to almost zero. Bottom substrate and other physical features will also impact the range. This is therefore a challenging method to use, and requires thorough testing of ranges in the study area and planning to ensure that essential data will be collected.

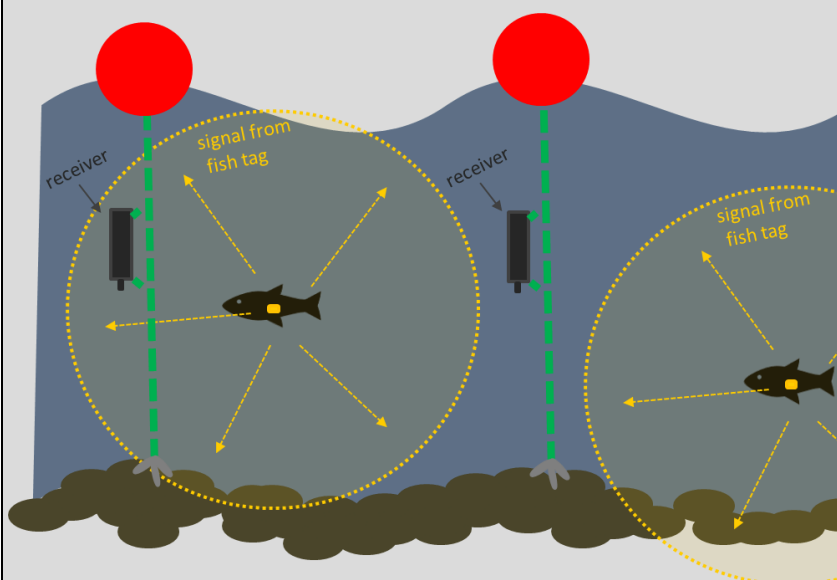


Illustration of two receivers that can receive signals from tagged fish. The receivers are here attached to a rope or wire that is anchored to the bottom and held upright by a floater on top, but other deployment methods can also be used. The fish tags (yellow) transmit signals in all directions (arrows) in a certain distance from the tag (yellow circle). Here, the left fish is detected by the left receiver, the right receiver detects no fish, and the right fish is not yet close enough to be detected.

Sensor tags provide extra information

Tags can be produced with sensors that for instance record at which water depth the fish is, temperature, light etc. This information is transferred as a code in the tag signal to the receiver when the fish is detected

Methods used to capture, tag and record fish are described in **figures 2-10** and **table 1**.



Figure 2. The fish species tagged in this study were asp, barbel, common carp, common nase, Pontic shad and vimba bream. Photos show two of the species; common nase (left photos) and barbel (right photos). Photos: Eva B. Thorstad.



Figure 3. Fish were captured for tagging in the Danube by fixed nets (mesh sizes of 20 to 60 mm) by Romanian and Serbian field teams and local fishers. Photos: Stefan Hont and Finn Økland.



Figure 4. Each fish was tagged with an acoustic transmitter (hereafter called tag). Three sizes of tags were used (9, 13 and 16 mm in diameter). The estimated life time of the tags was between 4.3 and 11 months. The smallest tags had the shortest life time. The largest tags were used for the largest fish. The two smallest tags had depth sensors, enabling recording of the fish swimming depth. Ping rate of the tags was random between 40 and 80 seconds. Photo: Eva B. Thorstad.

Figure 5. The fish were anaesthetised before tagging, 2-3 min in a bath with clove oil. Photo: Stefan Hont.





Figure 6. The tag was inserted into the fish body cavity via an incision during a surgical procedure that lasted a few minutes. After the tag was inserted, the incision was closed with sutures. Photos Eva B. Thorstad.



Figure 7. The fish were released back to the river quickly after tagging (few minutes), after they had recovered from anaesthetisation and could swim normally. A newly released barbel is shown in the photo. Photo Eva. B. Thorstad



Figure 8. In autumn 2019, the tagged fish were released at three sites; one site upstream of Iron Gate II (fish released during 19-20 October) and two sites below the dam (fish released 21-25 September and 17-21 October). In spring 2021, the tagged fish were released at five sites; one site upstream of Iron Gate II (fish released 25 March and 23-30 April) and four sites below the dam, of which three sites were in the main river and one site was in the Gogosu branch (fish released 28-29 April, 26 March, 7-10 May and 12 May in the main river and 29-30 April in the Gogosu branch). The fish released upstream of the dam were captured at Prahovo in Serbia, downstream of Iron Gate II, and transported in a tank with water upstream for release. All other fish were captured close to their release sites. Release sites are shown on the map in **figure 11**. Photos: Finn Økland



Figure 9. After release the fish were recorded by using stationary receivers, which automatically stored information on the date, time and identity of tagged fish when they were within the detection range of the receiver. For the tags with a depth sensor, the current swimming depth was also recorded. The upper left photo shows one of the receivers. Receivers were attached to wires, which were anchored to the bottom and held upright in the water column by floaters at the surface. Data were later downloaded to a computer. The study period in autumn 2019 lasted until the download of data from the receivers on 16 December 2019, and in spring 2021 until download of data on 16 June. Locations of receivers in the two study years are shown on the map in **figure 11**. Photos: Eva B. Thorstad and Finn Økland.



Figure 10. Tagged fish were also positioned by manual tracking, by using a boat searching for tagged fish with a portable receiver. Photos: Stefan Hont, Marija Smederevac-Lalic and Mirjana Lenhardt.

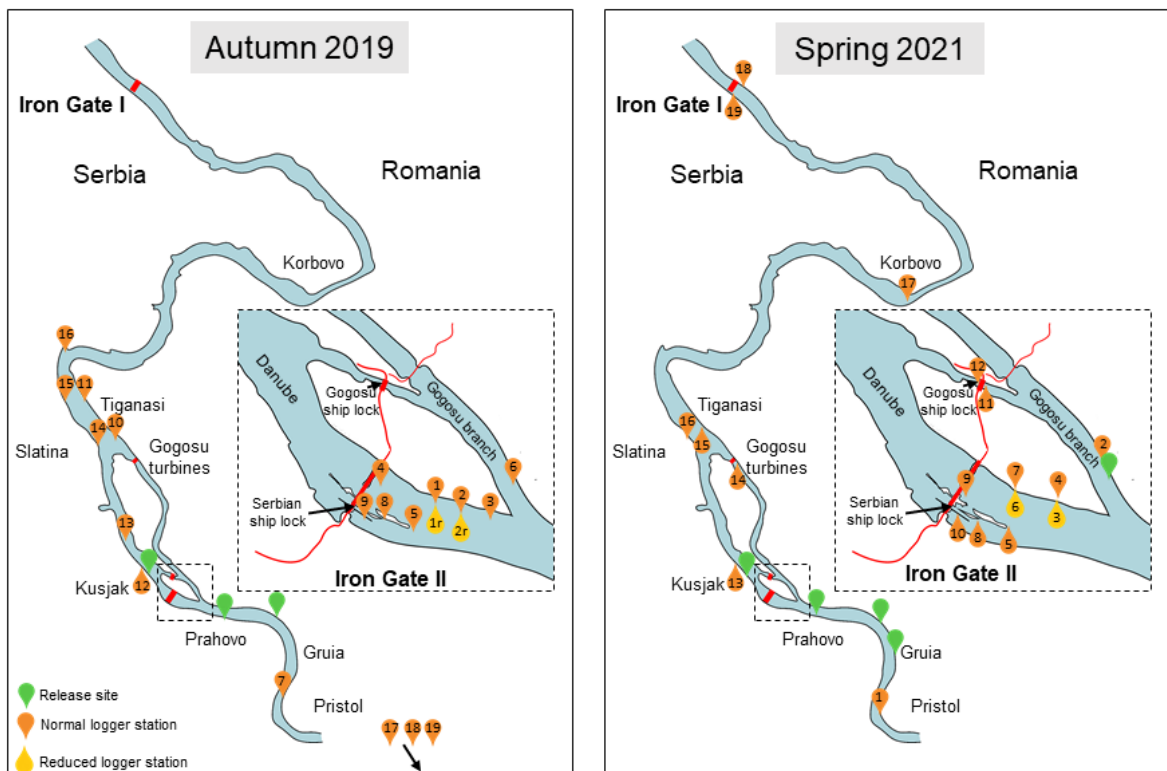


Figure 11. Map of the study area in the Danube at Iron Gate I and Iron Gate II, showing locations of release sites and acoustic receivers (logger stations) in autumn 2019 and spring 2021. The inset figure shows the Iron Gate II area and Gogosu branch in more detail. Two receivers had a purposely reduced range (reduced logger stations) compared to the normal receivers, to be able to locate the fish on a finer scale in that area. Each receiver is denoted with a unique identification number.

Table 1. Overview of tagged and released fish. Species, year they were tagged, total number of fish tagged, number of tagged fish released upstream and downstream of Iron Gate II (main river and Gogosu branch), number of tagged fish detected >24 or 48 hours after release, and fish body size.

| Species | Year | Total number of fish tagged | Number of fish released up-stream of Iron Gate II | Number of fish released down-stream of Iron Gate II in main river | Number of fish released down-stream of Iron Gate II in Gogosu branch | Number of fish detected > 24 h/48 h after release | Fish body size, total length in cm mean ± standard deviation (range) |
|-------------|------|-----------------------------|---|---|--|---|---|
| Asp | 2021 | 13 | 8 | 5 | - | 2/2 | 60.0* |
| Barbel | 2019 | 6 | 1 | 5 | - | 5/5 | 44.8 ± 4.5 (39.0-50.0) |
| | 2021 | 20 | 18 | - | 2 | 19/18 | 50.4 ± 9.0* (36.8-66.5) |
| Common carp | 2021 | 4 | 1 | 3 | - | 2/2 | 52.3 ± 12.6 (38.0-66.0) |
| Common nase | 2019 | 27 | 1 | 26 | - | 15/12 | 28.5 ± 3.0 (23.7-35.2) |
| | 2021 | 36 | 14 | 22 | - | 15/14 | 34.7 ± 1.6* (31.5 -39.0) |
| Pontic shad | 2021 | 7 | 4 | 3 | - | 0/0 | 32.0 ± 0.7 (31.0-33.0) |
| Vimba bream | 2019 | 28 | 19 | 9 | - | 25/24 | 30.4 ± 2.0 (27.5-35.0) |
| | 2021 | 44 | 18 | 14 | 12 | 42/42 | 36.2 ± 2.4 (31.0-41.0) |

Note 2021: *Total length not assessed from all fish (1/13 asp, 19/20 barbel, 33/36 common nase).

Results

Overview of tagged fish

A total of 185 fish were tagged; 61 in autumn 2019 and 124 in spring 2021 (**table 1**). Of the tagged fish, 101 were released 3-11 km downstream of Iron Gate II (87 in the main river and 14 in the Gogosu branch) and 84 in the reservoir < 2 km upstream of Iron Gate II.

The most common species among the tagged fish were vimba bream (72 fish) and common nase (63 fish), followed by barbel (26 fish) and asp (13 fish) (**table 1**). Small numbers of Pontic shad (7 fish) and common carp (4 fish) were also tagged.

Of the tagged fish, 151 (82%) were recorded after release. Some fish (32) were only recorded during the first two days after release, but 119 fish (64%) were recorded for more than two days after release (**table 1**). It was expected that not all fish would be recorded for long time periods after being released since each fish could only be recorded when they were within the detection range of a receiver. The Danube is a large river system, with the number of deployed receivers covering a relatively small area of the river. Additionally acoustic noise in some areas may reduce the detection range of certain receivers, thus fish had the potential to inhabit river areas beyond the receivers' detection range.

Based on the signals created by the acoustic tags, the receivers had received and stored a total of 475 444 detection events from tagged fish during the two study periods. Each fish was recorded by an average of 2 917 detection events across approximately 5 - 6 different receivers.

Overview of fish movements

Of the 101 fish released downstream of Iron Gate II, 48% moved in an upstream direction from their release site and were recorded by receivers below Iron Gate II (**figure 12, appendix 1** for species-specific details on fates and movement directions of tagged fish during autumn 2019 and spring 2021, **appendix 2** for examples of individual movement patterns). For 23% of the fish, it took less than 24 hours to reach this area, whereas the remaining fish took between 1.1 to 49 days to be recorded by receivers below Iron Gate II.

Both years, approximately half of the fish released downstream of Iron Gate II visited the lower part of the Gogosu branch, where the Gogosu branch enters the main river (**figure 12**). In 2019, receivers were only deployed in the lower part of the Gogosu branch (**figure 11**), and thus it could not be determined whether or not some fish moved further upstream in the Gogosu branch. In 2021, when receivers were deployed at the Gogosu ship lock and dam, several of the fish were shown to move further upstream in the Gogosu branch. Approximately one-third of the tagged fish were recorded below the Gogosu ship lock, and 15% of the tagged fish moved all the way to the upper part of the Gogosu branch and were recorded below the Gogosu dam (**figure 12**).

No tagged fish released downstream of Iron Gate II were observed in the reservoir upstream of Iron Gate II (**figure 12**). This means that no tagged fish passed the Iron Gate II dam or Gogosu dam in an upstream direction. Further, no tagged fish passed the Serbian ship lock at Iron Gate II or the Romanian ship lock at the Gogosu branch in an upstream direction.

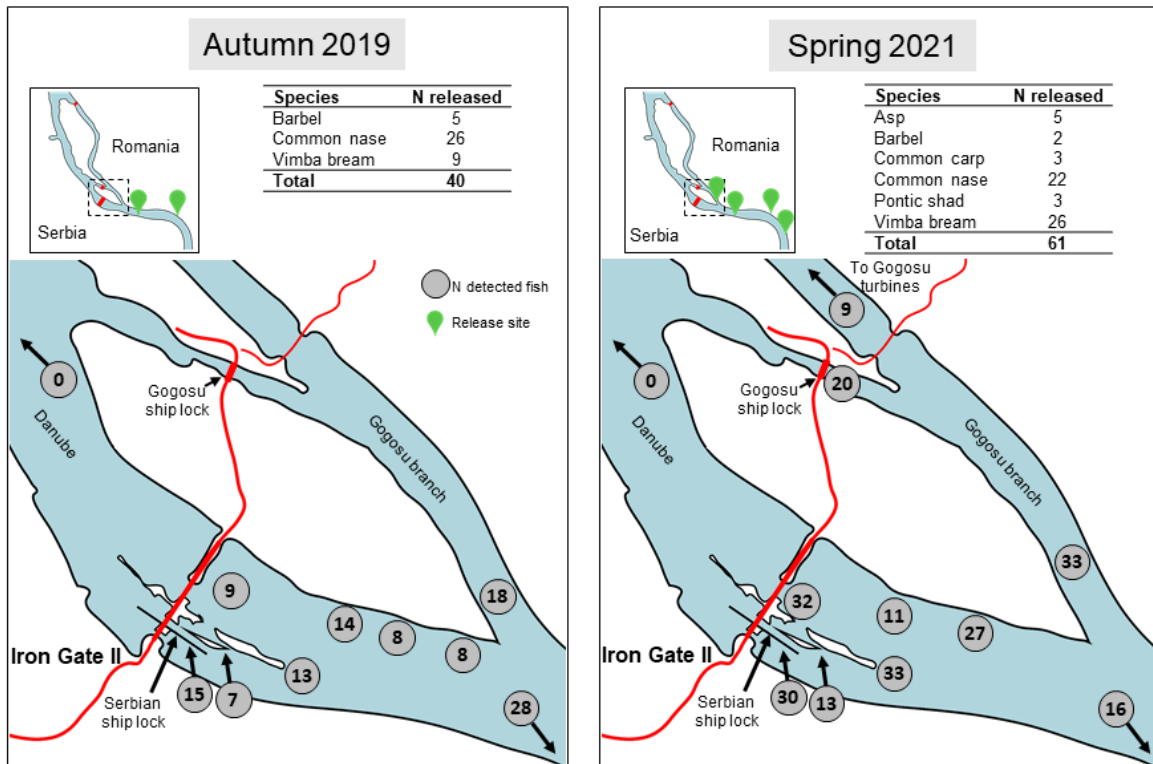


Figure 12. Overview of number of tagged fish recorded at different sites (grey bubbles) among fish released downstream of Iron Gate II during autumn 2019 and spring 2021 (all species combined). The tables provide an overview of the number of tagged fish released downstream of Iron Gate II each year. The map shows the area at Iron Gate II in detail, and the red line is the road between Romania and Serbia crossing over the Gogosu branch and ship lock and Iron Gate II. The release sites downstream of Iron Gate II are shown in the inset map, which shows a larger area of the river. For simplicity, many of the grey bubbles represent recordings at several nearby receivers combined (for map of all receivers, see **figure 11**). The three grey bubbles with an arrow pointing out of the map in an upstream or downstream direction show number of fish recorded at receivers outside the map. The bubble with an arrow in the Gogosu branch in 2021 shows the number of fish recorded at the receiver below the Gogosu dam (no receiver at this site in 2019).

The majority of tagged fish released in the reservoir upstream of Iron Gate II moved away from the Kusjak release site, and either moved further upstream in the reservoir (49% of the tagged fish), or in a downstream direction (48% of the tagged fish) and were recorded downstream of Iron Gate II (**figure 13, appendix 1**). In autumn 2019, nine fish moved to areas below the dam (14% of the fish released upstream), whereas in spring 2021, 40 fish moved to areas below the dam (about two-thirds of the fish released upstream).

Fish could have moved from the reservoir to areas downstream of Iron Gate II via several routes; through the Iron Gate II dam, through the Serbian ship lock at Iron Gate II, through the Romanian ship lock at Gogosu, or through the Gogosu dam. Passage through the dams either means through the turbines in the dam, or with spill water if there have been periods with high water discharge and release spill water (we do not have available data of whether there have been release of spill water).

In autumn 2019, one of the three fish (a vimba bream) that moved from the reservoir to areas downstream of Iron Gate II was first recorded at receiver no. 3 (Romania; 4.5 days after release; for receiver locations, see **figure 11**), with the other two downstream moving fish (a vimba bream and a barbel) being first recorded by receiver no. 5 (Serbia; 0.1 and 0.3 days after release, respectively). As these fish were first detected on receivers that were not immediately

downstream of Iron Gate II, it is difficult to determine whether they moved down through the Serbian ship lock or the Iron Gate II dam on the Serbian or Romanian side.

In spring 2021, 12 fish of the 40 fish that moved downstream of Iron Gate II (6 vimba bream, 3 barbel, 2 Pontic shad, 1 common nase) first moved upstream in the reservoir to Tiganasi and/or Slatina or above, before moving downstream to areas below Iron Gate II. The remaining 28 fish moved downstream of Iron Gate II within an average of 1.5 days after release (range: 1 minute-8.7 days). The majority (80%) of descending fish were first detected at receivers on the Serbian side of the Danube (receiver no. 5, 8 and 10), with the receiver below the Serbian ship lock (receiver no. 10) being the most common point of first detection below IGII (45%; 18/40 fish). The remaining 20% of fish were first detected by Romanian receivers (no. 7 and 9) downstream of the Iron Gate II (**figure 11**).

No fish moving downstream from the reservoir were first detected by the receiver immediately downstream of the Gogosu ship lock receiver (receiver no. 11; **figure 11**). However three fish were first detected below Iron Gate II at the receiver located at the base of the Gogosu branch (2 barbel, 1 vimba bream; receiver no. 2; **figure 11**). Whether these fish passed undetected through the Gogosu ship lock or turbines in the dam could not be determined. For fish that moved from the reservoir to areas downstream of Iron Gate II, it is not possible from the present study design to determine whether they survived the passage or not.

For details on the fish that moved upstream in the reservoir, see separate chapter below.

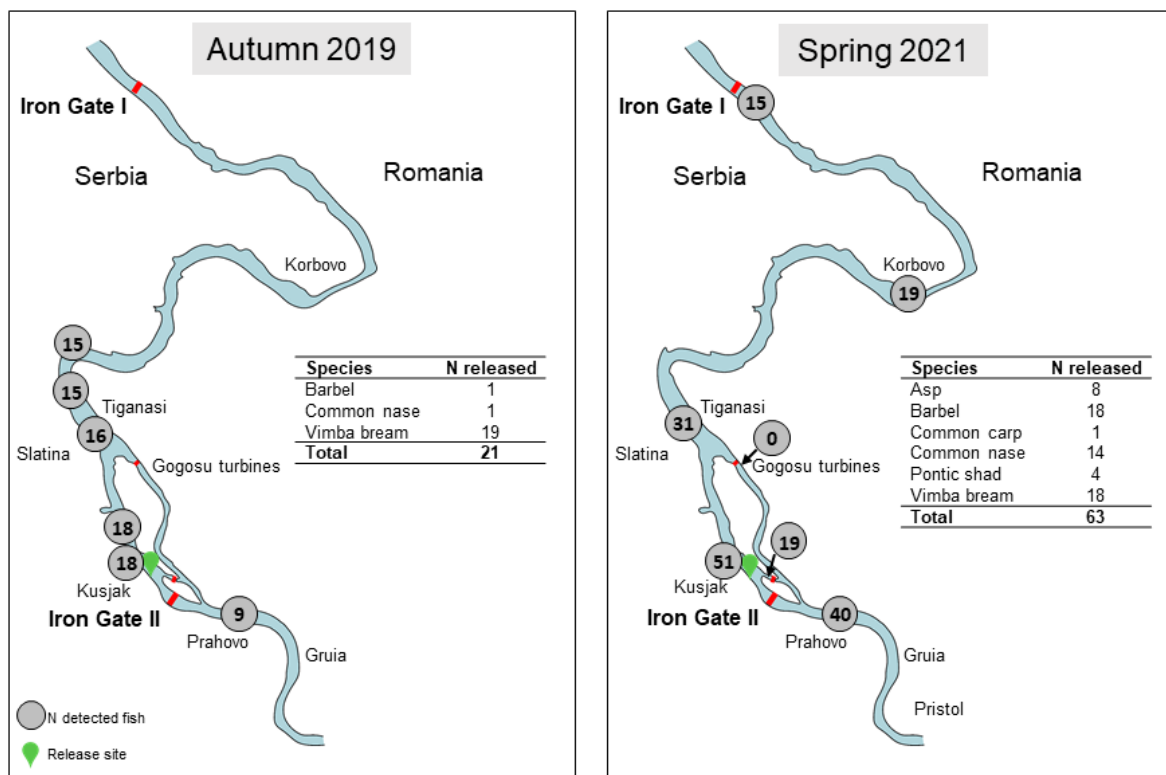


Figure 13. Overview of number of tagged fish recorded at different sites (grey bubbles) among fish released in the reservoir upstream of Iron Gate II in autumn 2019 and spring 2021 (all species combined). The tables give an overview of the number of fish released in the reservoir each year. The location of Iron Gate II including the Serbian ship lock, the Gogosu ship lock, and the Gogosu dam are indicated with red lines across the river. For simplicity, many of the grey bubbles represent recordings at several nearby receivers combined (for map of all receivers, see **figure 11**). The grey bubble below Iron Gate II shows the number of fish recorded below the dam, all receivers below the dam combined. If a fish had passed a site without being recorded by the receivers at that site, as indicated by recordings at receivers located further upstream, it was included as recorded at that site.

Behaviour at Iron Gate II

For the fish released downstream of Iron Gate II that were recorded in the area below the dam (*i.e.*, between the dam and the confluence with the Gogosu branch), there was no clear pattern to which side of the river they preferred to move along (**table 2**). Only a few fish were recorded either exclusively on the Romanian (4 fish) or Serbian (7 fish) side of the river. Most fish (43 fish) were recorded by receivers on both sides of the river.

Table 2. Overview of recordings below Iron Gate II for fish released downstream of the dam, shown as number of fish recorded by receivers on the Romanian and Serbian side of the river only, and by receivers on both sides of the river. The analysis is based on recordings by receivers between the dam and the confluence with the Gogosu branch. For 2019, receivers no. 1, 1r, 2, 2r, 3 and 4 were on the Romanian side of the river, and receivers no. 5, 8 and 9 were on the Serbian side (receiver locations are shown on the map in **figure 11**). For 2021, receivers no. 3, 4, 6, 7 and 9 were on the Romanian side of the river, and receivers no. 5, 8 and 10 were on the Serbian side.

| Sample period | Species | Serbia only (number of fish) | Romania only (number of fish) | Serbia and Romania (number of fish) | Total number of fish |
|---------------|-------------|---------------------------------|----------------------------------|--|----------------------|
| Autumn 2019 | Barbel | 0 | 0 | 2 | 2 |
| | Common nase | 5 | 2 | 5 | 12 |
| | Vimba bream | 0 | 0 | 5 | 5 |
| | Total | 5 | 2 | 12 | 19 |
| Spring 2021 | Asp | 0 | 0 | 3 | 3 |
| | Barbel | 0 | 0 | 2 | 2 |
| | Common carp | 0 | 1 | 1 | 2 |
| | Common nase | 1 | 1 | 1 | 3 |
| | Vimba bream | 1 | 0 | 24 | 25 |
| | Total | 2 | 2 | 31 | 35 |

Behaviour in the reservoir between Iron Gate I and II

Three-quarters of the fish released in the reservoir above Iron Gate II in the autumn 2019, and half of the fish released in spring 2021 moved further upstream in the reservoir. All these fish were at least recorded as far upstream in the river as the receivers at Slatina and/or Tiganasi, ~11 km from the release site (**figure 13**). In 2021, when receivers were deployed further upstream than in 2019, and fish movements could be recorded also in the upper part of the reservoir, nearly one-third of the tagged fish were recorded at Korbovo, ~45 km from the release site, and one-quarter of the fish moved through the entire reservoir and were recorded at Iron Gate I, ~76 km from the release site (**figure 13**).

Behaviour at Iron Gate I

For the fish that reached Iron Gate I, there was no clear pattern to which side of the river they preferred to move along (**table 3**). All fish recorded in this area were recorded by receivers on both sides of the river.

Table 3. Overview of recordings of tagged fish below Iron Gate I. The analysis is based on the two receivers deployed downstream of Iron Gate I in 2021, of which one was on the Romanian side and one on the Serbian side of the river. Numbers of fish recorded by each receiver, or both receivers, are shown in the table.

| Species | Serbia only (number of fish) | Romania only (number of fish) | Serbia and Romania (number of fish) | Total number of fish |
|--------------|---------------------------------|----------------------------------|---|-------------------------|
| Barbel | 0 | 0 | 5 | 5 |
| Common nase | 0 | 0 | 1 | 1 |
| Vimba bream | 0 | 0 | 9 | 9 |
| Total | 0 | 0 | 15 | 15 |

Species specific behaviour

For barbel, common nase and vimba bream, which were the most common species among the tagged fish, we analysed their species specific behaviour in both autumn 2019 and spring 2021 in more detail (**figure 14 and 15**). We also analysed the behaviour of asp in more detail, but they were only tagged in spring 2021.

Among fish released downstream of Iron Gate II, individuals of all four species were recorded below the dam, and in the lower part of the Gogosu branch. There were no clear patterns to which side of the river tagged fish moved among any of the species. In the area below the dam, there were no clear patterns whether tagged fish were recorded on the side where the Serbian ship lock is located, or below the dam itself, among any of the species; there were individuals of all species recorded at almost all the receiver sites (**figure 14**).

In the spring 2021, when more receivers were deployed in the Gogosu branch, the results showed that some individuals of barbel, common nase and vimba bream moved all the way to the upper part of the Gogosu branch and were recorded below the dam (**figure 14**). A large part of the vimba bream (40% of the tagged fish) were also located below the Gogosu ship lock. One barbel, but no common nase, were recorded below the ship lock. Asp were not recorded to move upstream in the Gogosu branch, neither to the ship lock nor to the dam.

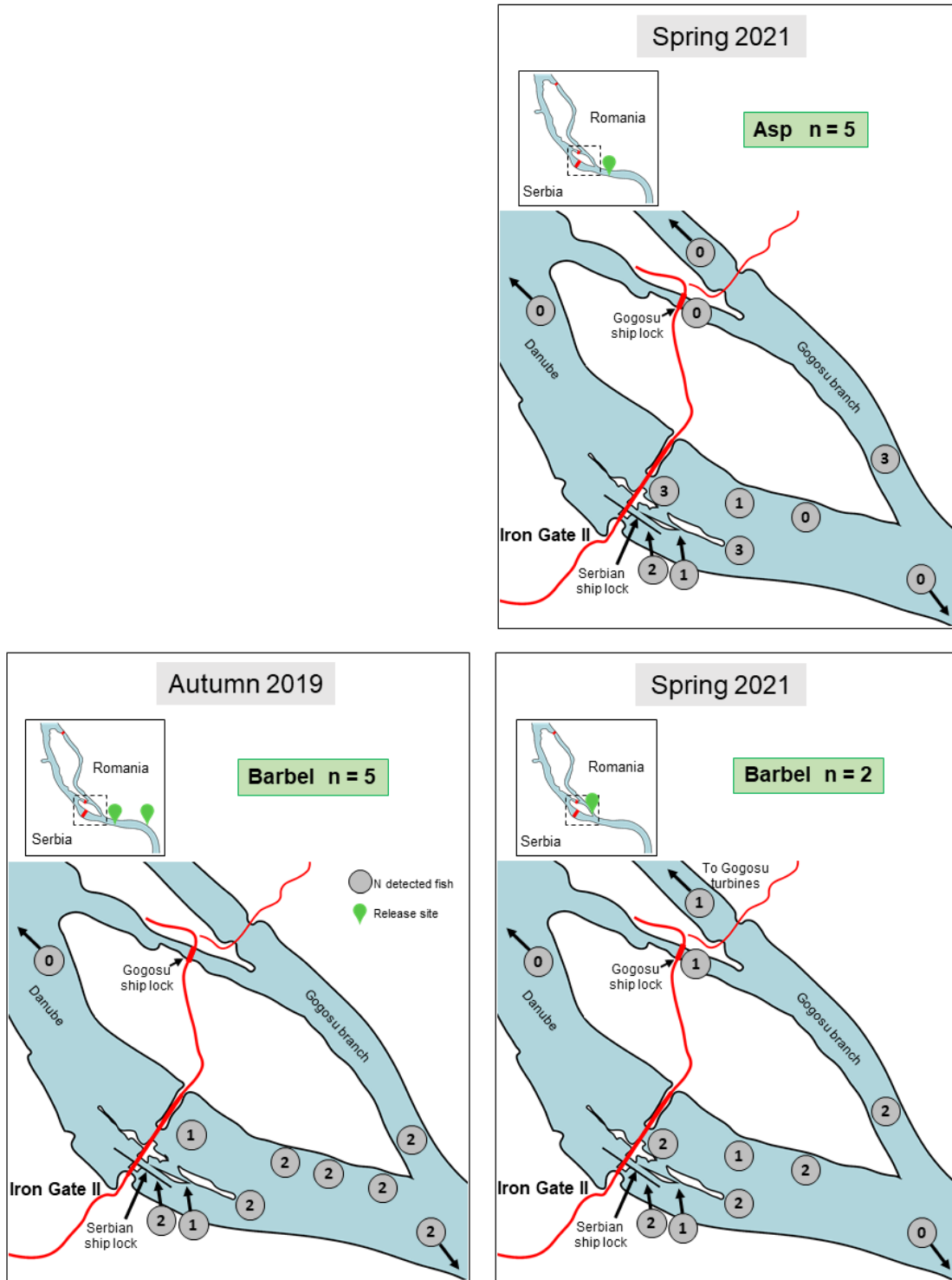


Figure 14. Continues on next page, see figure legend below.

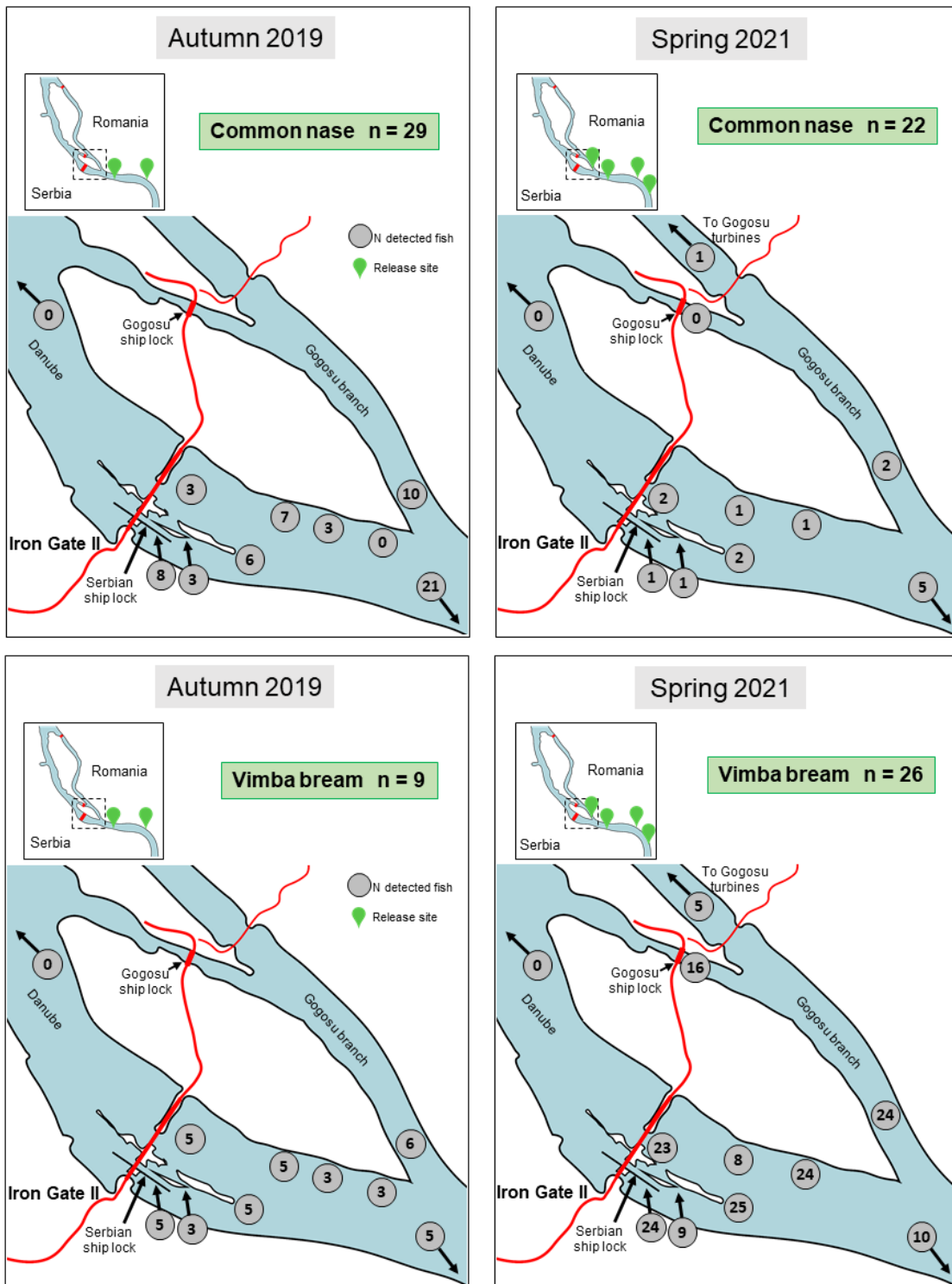


Figure 14. Overview of number of tagged fish recorded at different sites (grey bubbles) among fish released downstream of Iron Gate II in autumn 2019 and spring 2021 for asp, barbel, common nase and vimba bream in separate panels (asp were only tagged in 2021). The map shows the area at Iron Gate II in detail, and the red line is the road between Romania and Serbia crossing over the Gogosu branch and ship lock and Iron Gate II. The release sites downstream of Iron Gate II are shown in the inlet map, which shows a larger area of the river. For simplicity, many of the grey bubbles represent recordings at several nearby receivers combined (for map of all receivers, see **figure 11**). The three grey bubbles with an arrow pointing out of the map in an upstream or downstream direction show the number of fish recorded at receivers outside the map. The bubble with an arrow in the Gogosu branch in 2021 shows the number of fish recorded at the receiver below the Gogosu dam (no receiver at this site in 2019).

Barbel and vimba bream were the two species with the most pronounced upstream movements in the reservoir after being released above Iron Gate II (**figure 15**). In spring 2021, 72% of barbel moved at least as far upstream as Slatina and/or Tiganasi, ~11 km from the release site (**figure 15**) within 0.3-8 days after being released. Five barbel (27%) were observed to continue to move 76 km upstream through the entire reservoir and were detected at Iron Gate I between 2.2 -15 days after being released. Only one barbel was released in the reservoir in 2019, which was noted to move downstream below Iron Gate II.

Of the combined total of 37 vimba bream released in autumn 2019 and spring 2021, more than three-quarters of the fish moved upstream to Slatina and/or Tiganasi within 0.3-28 days (both years combined; **figure 15**). In 2021, when more receivers were deployed further upstream, half of the vimba bream were observed to move upstream through the entire reservoir to Iron Gate I (**figure 15**), with some individuals reaching this location after just 3.8 days (max. 17 days; 2021 only).

Asp and common nase were also observed to move upstream in the reservoir, but not to the same extent as barbel and vimba bream (**figure 15**). Of the eight asp released in the reservoir in spring 2021, one asp moved upstream to Slatina and/or Tiganasi in 0.2 days, whereas the seven other asp moved downstream to areas below Iron Gate II. Only one common nase was tagged in the autumn 2019, and this fish remained near the release site (**figure 15**). Among the common nase tagged in the spring 2021, 20% individuals moved 11 km upstream to Slatina and/or Tiganasi in 1.9-31 days. One out of 14 common nase released above Iron Gate II was detected to move upstream through the entire reservoir to Iron Gate I over a period of 34 days.

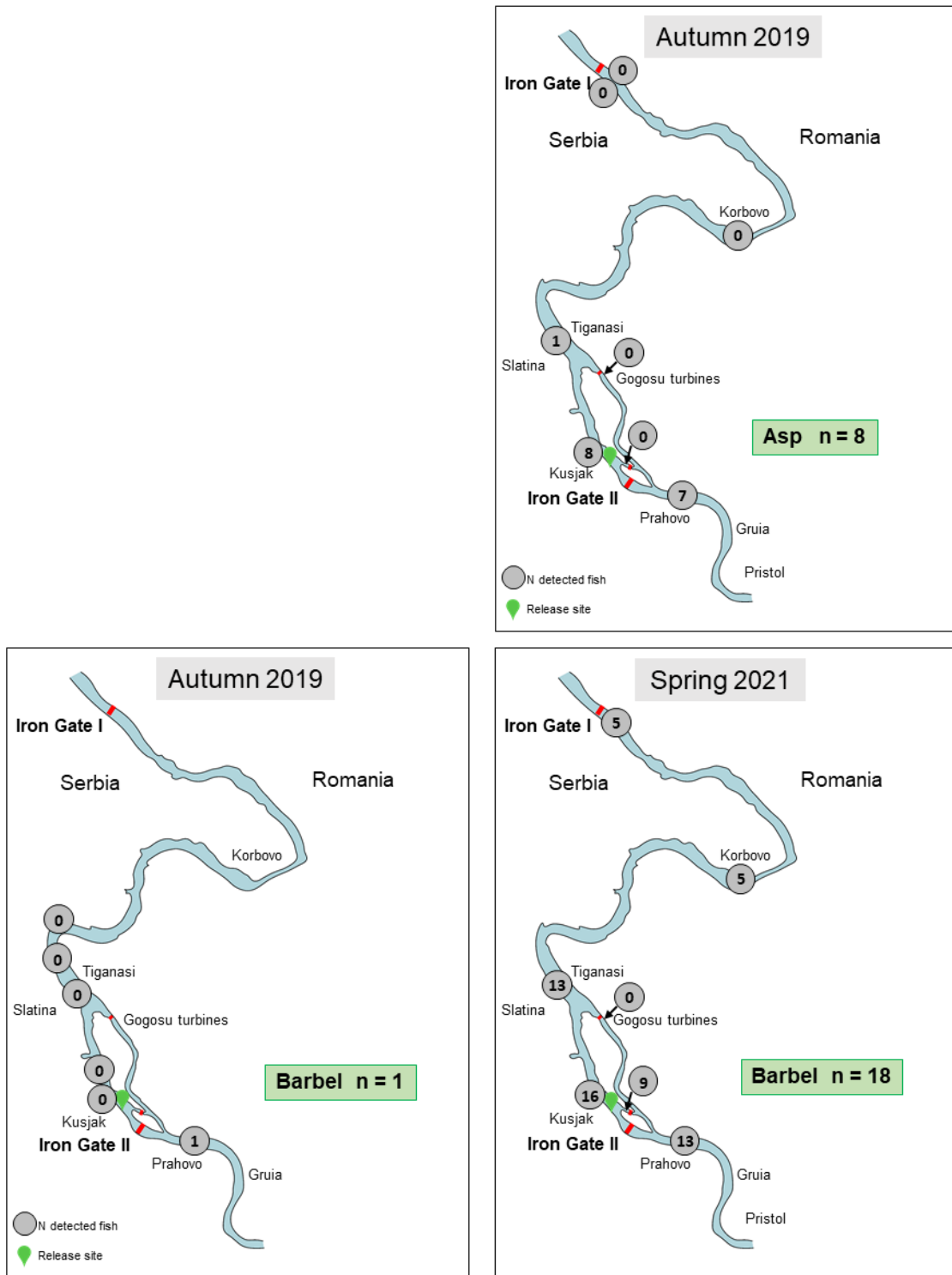


Figure 15. Continues on next page, see figure legend below.

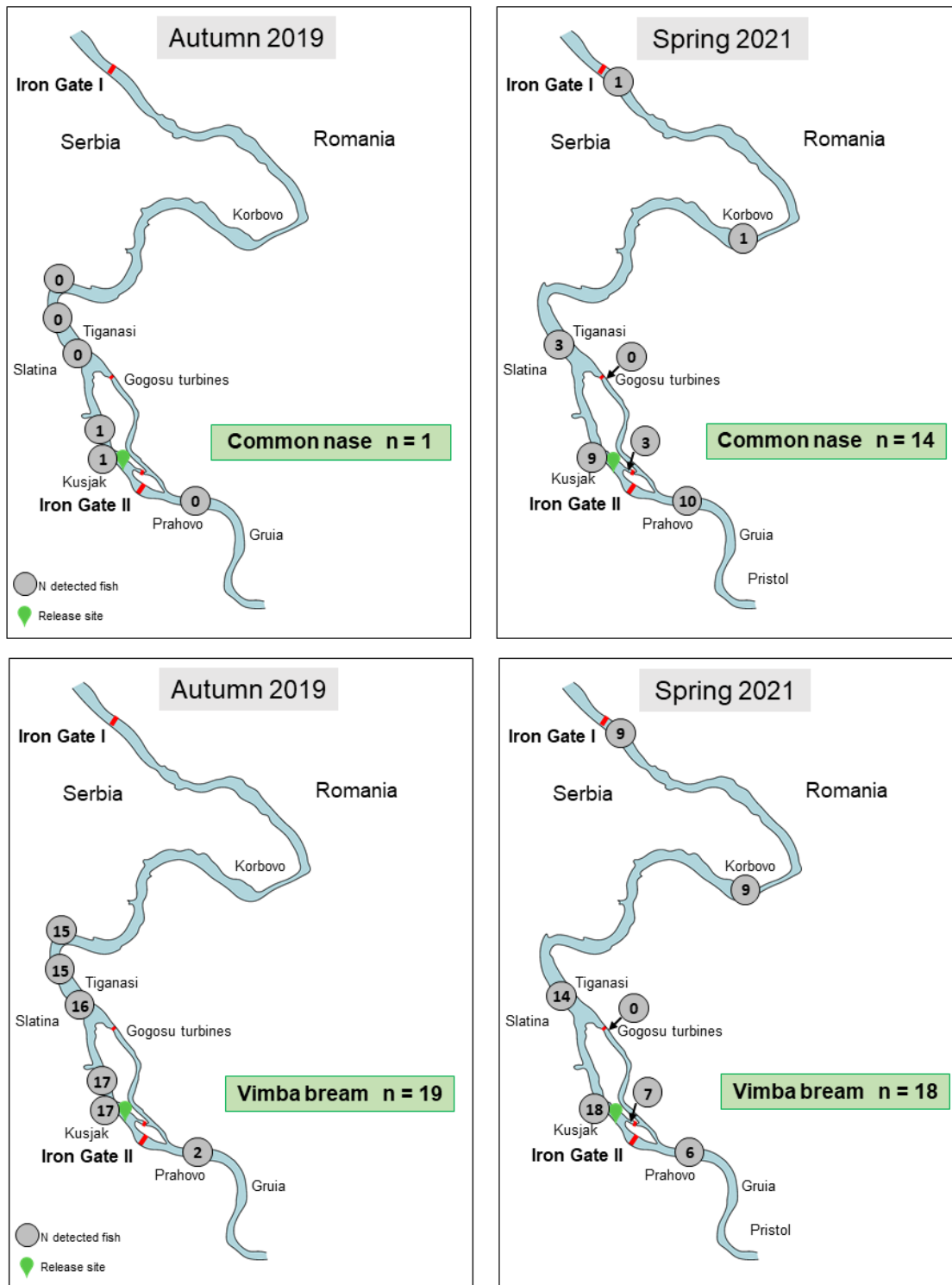


Figure 15. Overview of total number of tagged fish recorded at different sites (grey bubbles) among fish released in the reservoir upstream of Iron Gate II during autumn 2019 and spring 2021 for asp, barbel, common nase and vimba bream in separate panels (asp were only tagged in 2021). The location of Iron Gate II including the Serbian ship lock, the Gogosu ship lock, and the Gogosu dam are indicated with red lines across the river. For simplicity, many of the grey bubbles represent recordings at several nearby receivers combined (for map of all receivers, see **figure 11**). The grey bubble below Iron Gate II shows the number of fish recorded below the dam, all receivers below the dam combined. If a fish had passed a site without being recorded by the receivers at that site, as indicated by recordings at receivers located further upstream, it was included as recorded at that site.

Swimming depth

Barbel, common nase and vimba bream equipped with tags with depth sensors were swimming at mean depths of 3.5-3.9 m below Iron Gate II (**table 4**). Common nase were swimming at a shallower average depth below the surface in the reservoir than below Iron Gate II, whereas vimba bream were swimming at a deeper average water depth in the reservoir than below Iron Gate II. The deepest recording on any occasion by barbel was 12 m, by common nase 18 m and by vimba bream 22 m.

Table 4. Overview of the average (minimum-maximum) swimming depth (m) of barbel, common nase and vimba bream (2019 release only) at receivers below Iron Gate II and in the reservoir. Averages are based on averages for individual fish. The min and max values are the minimum and maximum depths registered by any fish in each group.

| Species | Depth m, average (minimum-maximum) | |
|-------------|------------------------------------|------------------|
| | Below Iron Gate II | Reservoir |
| Barbel | 3.6 (0.6-11.7) | NA |
| Common nase | 3.5 (0.3-17.8) | 1.9 (0.3-16.2) |
| Vimba bream | 3.9 (< 0.0-12.0) | 9.0 (< 0.1-22.3) |

Note: Iron Gate II (receiver number 1, 1r, 2, 2r,3, 4, 5, 8, 9); Reservoir (receiver number 10-16); see **figure 11** for locations.



Photos from field work, by Stefan Hont, Marija Smederevac-Lalic and Eva B. Thorstad.

Discussion

This feasibility study showed that we were able to successfully catch and tag fish with acoustic tags in the Danube, and follow their movements downstream of the Iron Gate Dam II, in the Gogosu branch, in the reservoir between the two dams, and up to the Iron Gate Dam I. The Danube is a border river between Romania and Serbia in the study area, with restrictions on crossing the river, but the study was effectively performed by the collaboration between Romanian and Serbian field teams carrying out the capture, tagging and tracking of fish on both sides of the river.

Valuable and informative results were obtained both during spring and autumn field seasons. The most abundant fish species among the tagged fish were asp, barbel, common nase and vimba bream, and the study showed that at least vimba bream, common nase and barbel can be captured and tagged in the study area both during spring and autumn seasons. In this study, asp were only captured and tagged in the spring, but it is possible that asp could also be included in studies in the autumn.

For many freshwater fish species, there is limited information on whether they are stationary fish living in smaller areas, or potamodromous fish, which perform migrations within freshwater bodies. Vimba bream, common nase, barbel and asp are all known to be migratory species, which can perform long-distance movements in freshwater. This was confirmed also for our study area, with many tagged individuals performing extensive movements, both below Iron Gate II, in the Gogosu branch and in the reservoir. Several barbel, common nase and vimba bream moved 76 km upstream from the release site in the lower part of the reservoir through the entire reservoir to Iron Gate I. Barbel and vimba bream were the species showing the most extensive movements, in terms of long-distance movements, and in proportions of the fish performing such movements. The study confirmed that there is large individual variation in movement strategies and behaviour within these species.

None of the tagged fish released downstream of Iron Gate II passed Iron Gate II and entered the upstream reservoir. This means that no tagged fish passed the Iron Gate II dam or Gogosu dam in an upstream direction. Further, no tagged fish passed the Serbian ship lock at Iron Gate II or the Romanian ship lock in the Gogosu branch in an upstream direction. It was expected that fish were not able to pass the dams with turbines in an upstream direction, but in theory, fish might be able to move through the ship locks in an upstream direction.

The fish released in the reservoir were captured downstream of Iron Gate II, and transported upstream in tanks with water for release. The study showed that some of these fish may move downstream again, but also that many of them were motivated for moving further upstream in the reservoir. Hence, this was a successful method to be able to record fish movements through the reservoir. For individuals passing the entire reservoir, the behaviour of these fish below Iron Gate I could also be studied. However, it should be pointed out that fish of the same species residing in the reservoir may to a larger extent migrate upstream than our tagged fish. This is because the fish in our study were captured and tagged below Iron Gate II, and we do not know the proportion that would be motivated to migrate further up the river among the fish residing in this area. Being captured and tagged may also reduce the motivation of some fish to migrate further upstream in a few days or weeks after release.

The detailed behaviour of the fish when approaching the Iron Gate II and Iron Gate I dams were studied by deploying several receivers with different detection ranges in the areas below the dams. The fish showed great individual variation in where they approached the dams, and most of the individuals were recorded by receivers on both sides of the river. Hence, a preference for moving along one particular side of the river was not identified for any of the species.

Studies of detailed behaviour of tagged fish can only be as fine-scaled as the receiver deployment allows. In the present study, a relatively limited number of acoustic receivers was

used, limiting the analysis of detailed behaviour below the dams. Further studies will be performed to increase the information of the detailed fish behaviour below the dams.

Iron Gate Dam I and II have obstructed the movements and migrations of many fish species in the Danube, including the large sturgeons, since the dams were constructed. Building fish passes in such areas requires information on the behaviour and migrations of many fish species with different life histories, body sizes and swimming capabilities. Obtaining such information is demanding. Most migratory species seem to prefer spawning in the area where they hatched. This so-called homing constitutes the most important motivation for fish to swim large distances upstream in rivers. A consequence of Iron Gate having been in place as a barrier to migration for many decades, large sturgeons caught below the Iron Gate II dam have never been in the area above the dam. The restricted spawning areas below the dam have just barely secured the survival of the populations in the Danube. However, these fish likely no longer have any homing motivation for trying to reach upper reaches of the river, and few of them are expected to actively search to find a passable route below Iron Gate II Dam. With functional fish passes at the Iron Gate dams, there might be a possibility to re-establish sturgeon migration past the dams

This might be different for smaller migratory fish species, like those included in the present study. Smaller migratory species spend their whole life cycles in the river, and with strong populations upstream of the dam, numerous individuals can most likely migrate downstream past the dam and turbines early in life. When maturing sexually they would likely be motivated to migrate back to upstream areas where they were hatched. Such fish may provide the model species that enable us to identify areas below the dam where migratory fish aggregate. Although the smaller riverine species may behave like the larger sturgeons in terms of their general response to water currents and turbulence, this cannot be certain. Verifying this requires a comparison of data from smaller species and large sturgeons. To some extent, the behaviour of large sturgeons has been recorded below the Iron Gate II dam. However, the number of tagged fish is too low to identify the main migratory routes and the behaviour below the dam. Nonetheless, this information will be valuable for a comparison with the behaviour of the smaller migratory species, which are abundant and can be caught and tagged in high numbers.

Appendix

Appendix 1

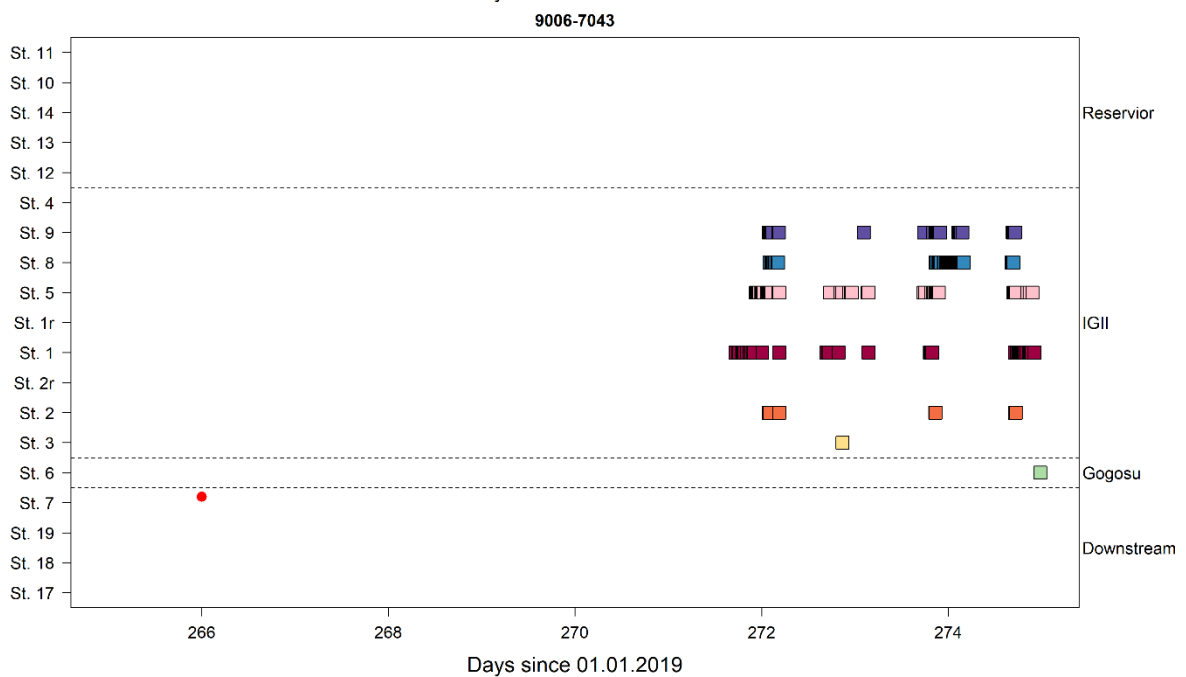
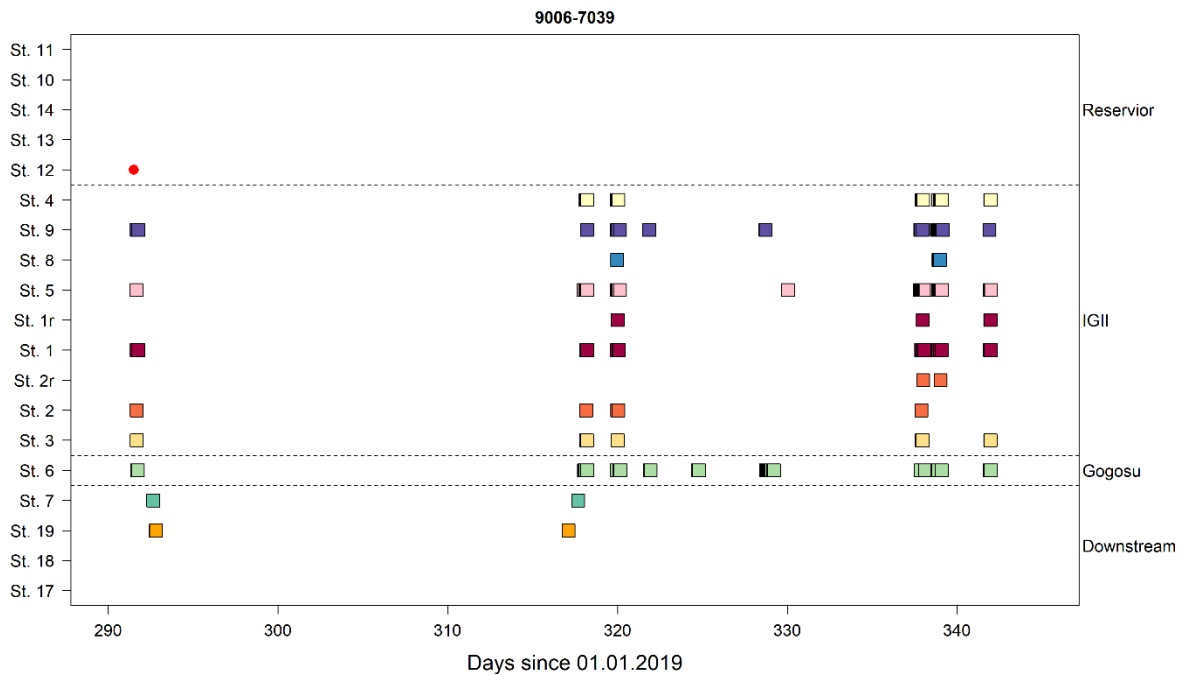
Overview of fish movement outcomes after release from locations upstream or downstream of the Iron Gate II dam for each tagged species in relation to season.

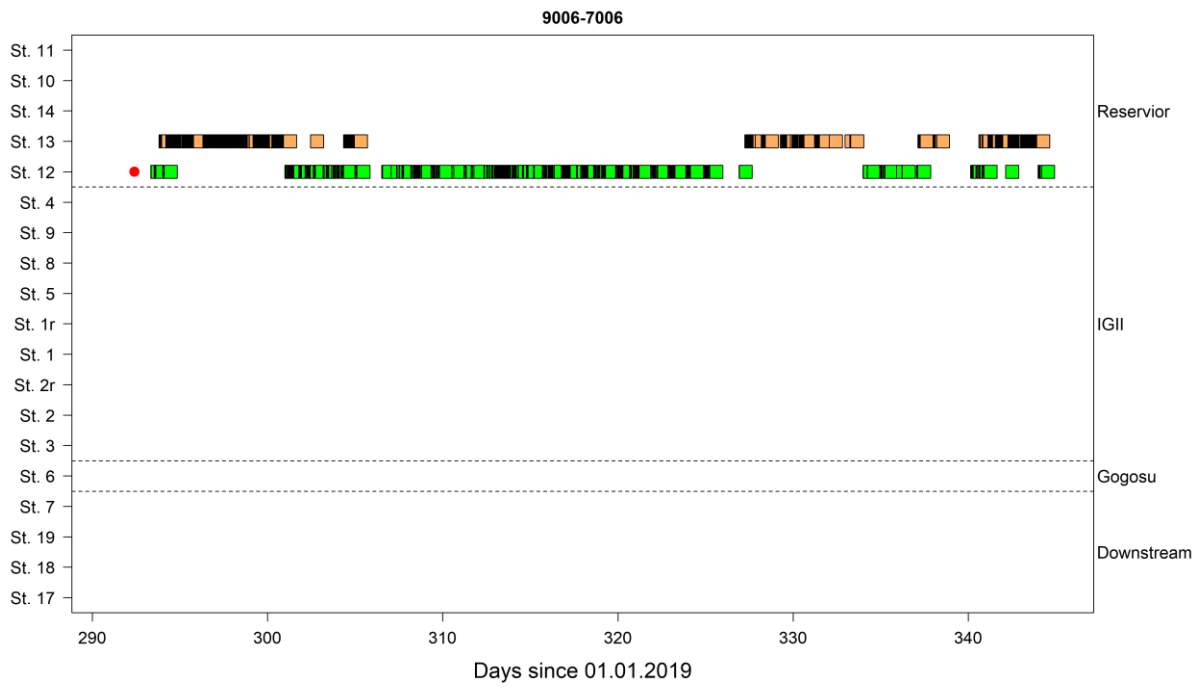
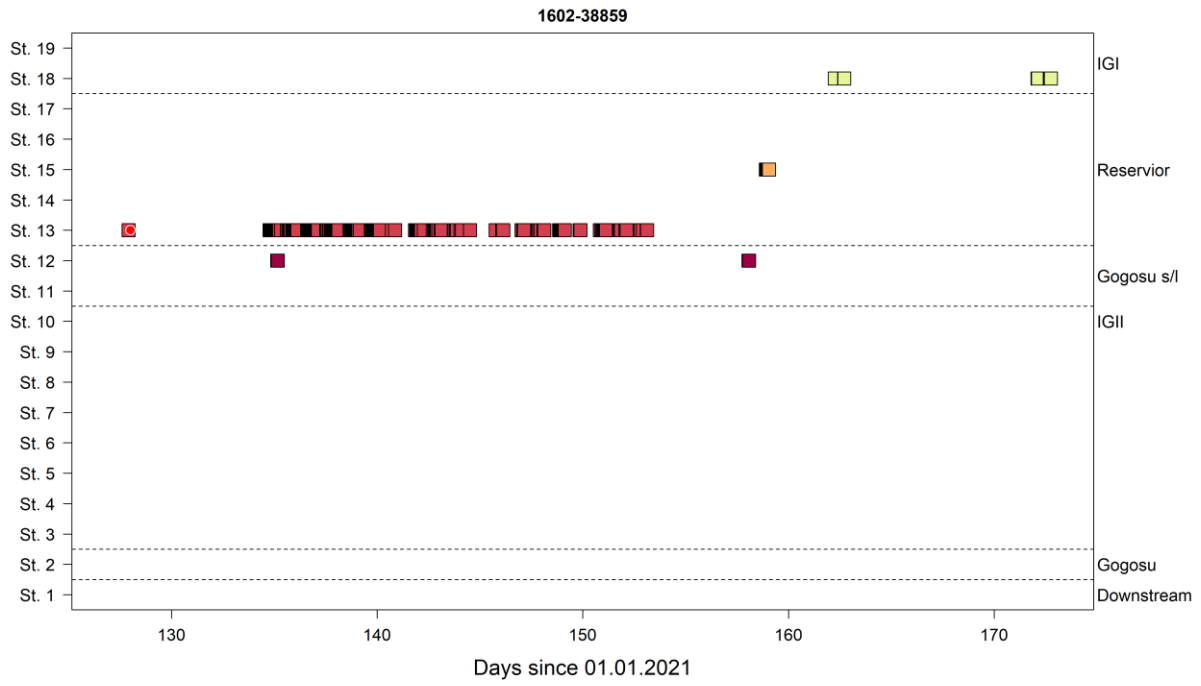
| Species | Season / Year | Iron Gate II release location | Number of released fish | Movement outcome after release (% of tagged fish) | | | |
|-------------|---------------|-------------------------------|-------------------------|---|------------|----------------|------------------|
| | | | | Not detected | Stationary | Moved upstream | Moved downstream |
| Asp | Spring 2021 | Upstream | 8 | 0 | 0 | 12.5 | 87.5 |
| | | Downstream | 5 | 40.0 | 0 | 60.0 | 0 |
| Barbel | Autumn 2019 | Upstream | 1 | 0 | 0 | 0 | 100 |
| | | Downstream | 5 | 20.0 | 0 | 40.0 | 40.0 |
| | Spring 2021 | Upstream | 18 | 0 | 0 | 72.2 | 27.8 |
| | | Downstream | 2 | 0 | 0 | 100 | 0 |
| Common carp | Spring 2021 | Upstream | 1 | 100 | 0 | 0 | 0 |
| | | Downstream | 3 | 33.3 | 0 | 66.6 | 0 |
| Common nase | Autumn 2019 | Upstream | 1 | 0 | 0 | 100 | 0 |
| | | Downstream | 26 | 11.5 | 0 | 46.2 | 42.3 |
| | Spring 2021 | Upstream | 14 | 0 | 7.1 | 21.4 | 71.4 |
| | | Downstream | 22 | 0 | 0 | 42.9 | 57.1 |
| Pontic shad | Spring 2021 | Upstream | 4 | 0 | 0 | 0 | 100 |
| | | Downstream | 3 | 100 | 0 | 0 | 0 |
| Vimba bream | Autumn 2019 | Upstream | 19 | 0 | 0 | 10.5 | 89.5 |
| | | Downstream | 9 | 22.2 | 0 | 66.7 | 11.1 |
| | Spring 2021 | Upstream | 18 | 0 | 0 | 77.8 | 22.2 |
| | | Downstream | 26 | 3.8 | 0 | 96.2 | 0 |

Note: 'Moved upstream' (%) includes fish that moved both up- and downstream after release, whereas 'Moved downstream' includes fish that only moved downstream.

Appendix 2

Examples of detailed movement patterns of four individual fish (id numbers of each fish is given above each panel). Panel 1: vimba bream released in the reservoir in 2019. Panel 2: barbel released below Iron Gate II in 2019. Panel 3: common nase released in the reservoir in 2021. Panel 4: vimba bream released in the reservoir in 2019. The red dot shows the release site and date, The squares show all the recordings of the individual fish at different receivers (station numbers on the vertical axis to the left corresponds to receiver numbers in **figure 11**). The receivers are divided into the areas downstream of Iron Gate II, the Gogosu branch, receivers immediately below Iron Gate II, and the reservoir (vertical axis to the right). The time line is given as day numbers since 1 January (horizontal axis).





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