Individual internship report

4ème année

Habitat assessment of juvenile Danube Salmon (*Hucho hucho*) in the River Pielach

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Table

I. Introduction

I.1. Context

Danube salmon (*Hucho hucho*, Linnaeus 1758), called Huchen in German, is a species belonging to the genus Hucho of the Salmonidae family. This salmonid is endemic to the Danube basin (Holčík et al., 1988). Seven distinct populations are still settled in Austria, Germany, Slovakia, Ukraine, Romania, Hungary, Slovenia, Bosnia and Serbia (Figure 1).

Figure 1 : Distribution of Hucho hucho, in orange, in Europe (IUCN, 2008)

Danube salmon can live up to 20 years, with an average life expectancy of around 15 years. It can grow up to between 130 and 140 cm in size and weigh up to 25 - 30 kg. The historically documented maximum size and weight of this species is 150 cm and 50 kg. However, it is almost impossible to come across individuals with these characteristics today (Pinter et al., in prep.). During its life cycle the Danube salmon uses different habitats. A habitat is defined in this report as an individual's living space. Adults of Danube salmon need flow velocity between 50 cm/s and 59 cm/s, a depth between 40 cm and 59 cm to spawn and a substrate composed by microlithal (20 to 63 mm) and mesolithal (63 to 200 mm) (Table 1). Larvae habitats are very different: the mean flow velocity is about 0.7 cm/s, the mean depth is about 20.8 cm and the substrate is composed by pelal (below 0.063 mm) and psammal (0.063 to 2 mm). Danube salmon juveniles prefer flow velocity between 10 cm/s and 50 cm/s, depth between 30 cm and 60 cm and substrate composed by microlithal. Danube salmon adults are found in deeper water and in habitat with flow velocity between 0 and 30 cm/s.

*Table 1 : Mean habitat characteristics of different Danube salmon life stages (*Ihut et al, 2014; Holzer et al, 1999; Holzer et al, 2011; Schöfbenker, 2018).

	Habitat characteristics		
Danube Salmon life cycle habitat	**Mean velocity**	**Mean depth**	**Substrate**
Spawning habitat	50-59 cm/s	40-59 cm	Micro- and Mesolithal
Larvae and juvenile habitat	About 0,7 cm/s	About 20,8 cm	Pelal and Psammal
Juvenile habitat	> 10-50 cm/s	>30-60 cm	Microlithal
Adult habitat	> 0-30 cm/s	>120-150 cm	Microlithal

As it is shown, the ecology of the Danube salmon depends on the different available habitats in the river but also on the water temperature. Spawning begins when the water temperature is around 6°C over the long term (Guttmann, 2006). Males and females form pairs before spawning (Esteve et al, 2009). Reproducers use tail movements to dig the mineral substrate before spawning. Danube male salmon are sexually mature between 3 and 4 years of age, while females are sexually mature at 4 to 5 years old (Freyhof & Kottelat, 2008).

Danube salmon is at the top of the food chain in the rivers. That is why this specie is considered as a flagship specie for ecological monitoring on those rivers (Estes et al, 2011). However, since 2008, Danube salmon has been classified as endangered by the IUCN (Freyhof & Kottelat, 2008), and the last populations of this species are declining (Effenberger et al., 2021). Numerous pressures such as habitat degradation (Höfler et al, 2017), overexploitation (Witkowski et al, 2013), loss of genetic diversity (Horreo et al, 2014), climate change (Melcher et al, 2016) and fragmentation of migration paths by dams (Freyhof et al, 2015) are causing *Hucho hucho* population to decline.

One of the last viable populations of Danube salmon is settled in the Pielach River, in Austria (Schmutz et al, 2002). This river is located in lower Austria and is a tributary of the Danube (Figure 1). The river is 67 km long and takes its source in Annaberg (Wasserstandsnachrichten Und Hochwasserprognosen, n.d.).

Figure 2 : Location of Pielach in Austria. Inset B shows the study area. ©Emmanuelle Waguette & Illona Doyon

That is why it is important to know well the ecology of these species, particularly during their most vulnerable life stage, like the larvae stage (Hauer, 2004). Indeed, this life-stage has a mortality rate of 95% over the first year (Bajić et al, 2015). Moreover, the habitat degradation being one of the Danube salmon populations decline cause, it is important to characterize the larvae habitat.

However, knowledge of the ecological demands of *Hucho hucho* is very poor. Indeed, the Web of Science does not have more than 28 publications on Danube salmon, even though it is not only an endangered species but also an important one in aquatic ecosystems. While almost all of these publications highlight efforts to protect this species, only a very few of them study the ecology and biology of the Danube salmon. Therefore, it seems necessary to improve knowledge of the Danube salmon and its population, particularly larval habitats (only one study in 2016 on the Pielach), in order to establish new basis for future management actions for the Danube salmon populations present in the Pielach.

I.2. Motivations

During this internship, my main purpose was to discover the field work and to know how to deal with it because I think that skill is very important to understand the data. This project was an opportunity to acquire practical skills. Moreover, the main topic of this project was a flagship fish, and an endangered one. Thus, I always wanted to participate to the conservation of habitats and species. The data that I collected could be used not only to improve the ecological knowledge about Danube salmon, but also to enable future management actions and protection measures. That is why I was very interested and motivated. I think my work would help the Danube salmon conservation. Indeed, a better comprehension of the larval ecology could bring arguments to restore aquatic environments or to settle management and protection measures along the Pielach.

Another personal motivation was to work with researchers in another country. Indeed, I had the opportunity to do an internship in France at the Reunion University a few years ago and was wondering if work methods were similar abroad or if other methodologies would be used, so I could learn from different experiences.

I.3. Objectives and missions

The aim of this project is to provide a basic knowledge about Danube salmon in the Pielach, focusing on larvae habitat. Indeed, Danube salmon Pielach population has been declining for the last couple of years so the reproduction success is important for the viability of the population. These acquired knowledge will be used for future management actions.

This project was divided in different parts. First, a spawning redds mapping was realized to understand were the Danube salmon spawned. In a second time, where I started to intervene, we have to do a larvae mapping and to characterize their used habitats. Then, we describe the available habitats present in the Pielach, both in the full flow and the residual flow. We also studied the compactness in this both parts of the river. The last step of this project is to evaluate the Danube salmon stock in the Pielach. This part will be done during the winter of 2023.

So, my missions during this project were searching the late spawning fish, describing the used habitat and the available habitat of larvae, comparing the substrate between the full flow and the residual flow and, to finish, comparing the current collected data with those collected in 2016.

II. Presentation of the host organization

The Institute for Hydrobiology and Water Management is part of the BOKU University (funded in 1872) in Vienna, Austria. Its main focus is the study of the functions, processes and structures of aquatic ecosystems and their surrounding environment. It is therefore a research and teaching center for all subjects relating to aquatic ecosystems, such as biodiversity, hydrology, biotic interactions and the impact of climate change on these environments, for example.

The institute is divided into 6 working groups:

- Biochemistry and ecohydrology of river landscapes (BIGER)
- Integrative modelling and management of aquatic ecosystems (IMMA)
- Ecology, diversity and assessment of benthic macroinvertebrates (RHEOPHYLAX)
- Socio-ecological management of river landscapes and historical water research (SEM)
- Fish ecology, sustainable fisheries and aquaculture (FISH)
- International Research and Education Network for the Sustainable Management of Aquatic Ecosystems in the Global South (IPGL)

This internship takes place in the FISH working group. Their researches focus on the ecology of fish, such as the Danube salmon, and the effects of climate change on the ecology of these fishes. These two topics are just two examples of the range of researches carried out by this ten-strong working group.

Their main role is to carry out researches on the full spectrum of fish ecology, whether in natural environments or those shaped by human activity. The main objective is to use this new knowledge to protect species and restore viable populations of endemic fish species such as the *Hucho hucho*. This will involve establishing methods and strategies in order to implement a sustainable management of fish stocks, harmonizing the conservation of these species with recreational fishing, and studying the impact of climate change on these species.

III. Missions and research questions

My first mission was to look for late spawning fishes and some first larvae along the study area in order to complete the spawning redds mapping. This task was important for the project as an exceptional flow event was settled in the middle of April. This flow event corresponded to a biennial discharge in the Pielach and reached almost 130 m^3/s. This flood interrupted the Danube salmon spawning and could also entail a high mortality rate of the Danube salmon eggs and larvae. All the project depended on finding some late spawning fishes and larvae as the main purpose was to describe the larvae habitats.

As some larvae survived and have been found, the project was able to continue. So, my second mission was to map all the larvae found in the study area and describe their habitats. This part was very important as the data would be used to understand the main characteristics of the larvae used habitats compared to the available habitats which could be found in the study area in the Pielach. Moreover, this data could be used to compare the characteristics of the used habitats found in 2016 and 2023. Then, we had to describe the available habitats. This collect of data enables to complete the knowledge gathered in 2016. Indeed, more transects were done during this project than in 2016.

During the larvae mapping, a surprising result was discovered. Indeed, a large difference between the full flow and the residual flow in terms of larvae presence was highlighted. This difference, which will be presented later in this report, had a link with the stability of the substrate. That is why we studied the compactness of the substrate too.

Finally, I had to use some statistical software like Excel, R and QGIS to analyse the data collected.

These different missions enabled us to answer the internship's main research questions:

1. How are the Danube salmon larvae habitats characterised?

    Hypothesis 1 (HYP1): The larvae habitats found in 2023 shared the same characteristics that those found in 2016.

2. As the Pielach is divided between residual and full flows, does it impact the survival of the Danube Salmon eggs or larvae?

    Hypothesis 2 (HYP2): The substrate in the full flow is more stable than in the residual flow which allows a better protection of the Danube salmon eggs during exceptional flow events.

IV. Methodology

IV.1. Study area

The study covered the downstream part of the river Pielach, from its confluence with the Danube to the Weinburg dam, i.e., more than 32km of survey (Figure 3). The Pielach has a Strahler stream order of 4 (Table 2). Its source is at an altitude of 800 m, and the river flows into the Danube at 208 m above sea level. The Pielach has a slope of 0.884% and a catchment area of 590 km². It is mainly a lowland river. The river's mean annual flow is 6.46 m^3/s and its minimum monthly flow is 1.82 m^3/s.

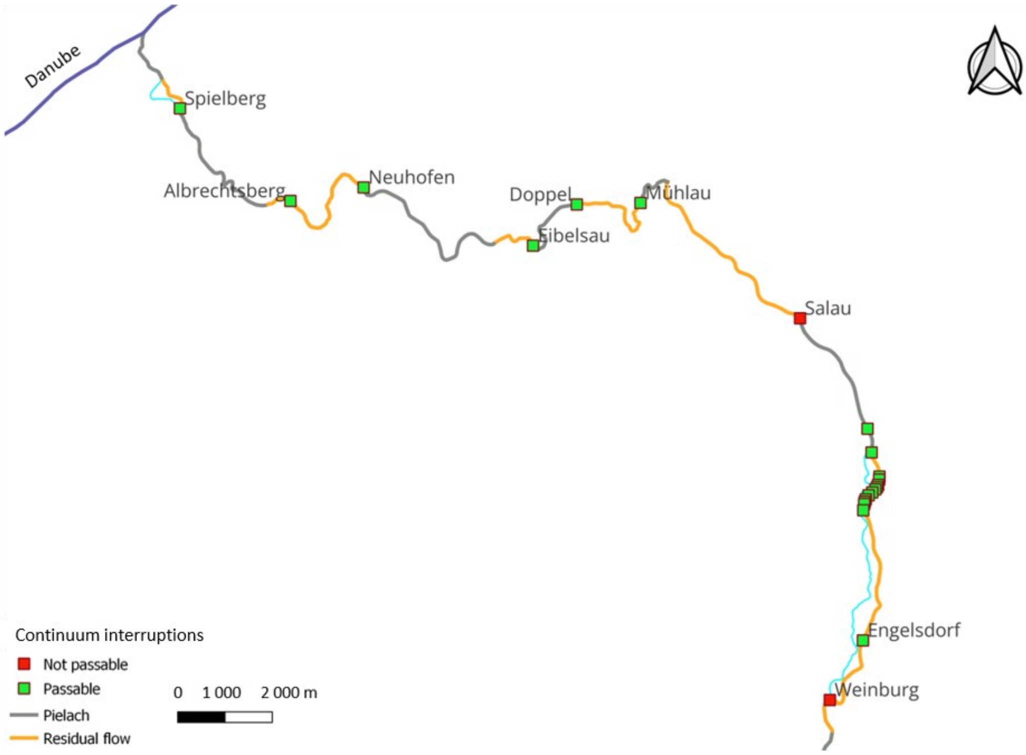

Figure 3 : Pielach river study area with continuum interruptions and residual flow section

Table 2 : Overview of the main characteristic of the Pielach river (Wasserstandsnachrichten Und Hochwasserprognosen, 2023).

River length	Source	Catchment	Flow regime	Stream order	MNQ	MQ	HQ1	HQ2	HQ10	HQ100
67 km	Annaberg	590 km²	Pluvio-nival	4	1,82 m^3/s	6,46 m^3/s	95 m^3/s	130 m^3/s	240 m^3/s	370 m^3/s

The Pielach has a pluvio-nival regime and two high-flow seasons: March to April and November to December caused by precipitation (Fink et al, 2000). Average annual precipitation is around 875 mm (Holzer, 2011).

The main fish families are cyprinids such as common barbel (*Barbus barbus*), nase (*Chondrostoma nasus*) and chub (*Squalius cephalus*). Among salmonids, the Pielach is home to Danube salmon (*Hucho hucho*), grayling (*Thymallus thymallus*), common trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*).

The Pielach watershed is mainly characterized by agricultural areas (Melcher & Schmutz, 2010). Conservation and restoration projects have been carried out, especially during the LIFE-HUCHEN project (1999-2004): riparian restoration, installation of fish pass and dam removal. In 2015, the Pielach was assessed as being in very good morphological condition between Weinburg and the Danube (Pletterbauer et al, 2015), even if 18 km of the river are a residual flow.

The residual flow is mainly created by hydropower dam. Indeed, a part of the Pielach is deviated to create hydroelectric power. As it is shown in the figure 3, the residual flow sections divide the Pielach in different parts in the same way as the weirs, artificial falls and rock ramps that are in the Pielach

(Table 3). As a result, the river is divided according to whether these obstacles can be crossed by fishes or not. There is a fish pass at the Salau weir, but no study demonstrated its effectiveness. However, in the opinion of some scientists, such as K. Pinter (personal communication), this fish pass is not very effective. This obstacle is therefore classified as not crossable by fishes.

These obstacles, whether they can be crossed or not, can lead to a reduction in exchanges between salmon from different parts of the river. This is why we can define 2 sub-populations (Table 3, ID 1 and 2) and 9 sub-reaches (Table 3: ID 3 to 11).

Table 3: Fish migration barriers divide the study area into two sub-populations and nine sub-reaches.

	ID	Continuum interruption	Type	Passability	Stretch length (km)	Residual flow (km)
Sub-population	1	Spielberg	Weir	No Passable	22,95	11,43
	2	Weinburg	Weir	No Passable	9,32	5,81
Sub-reach	3	Spielberg	Weir	Passable	1,82	0,91
	4	Albrechtsberg	Artificial fall	Passable	3,7	0,62
	5	Neuhofen	Weir	Passable	2,88	2,88
	6	Eibelsau	Weir	Passable	4,91	0,67
	7	Doppel	Weir	Passable	1,57	0
	8	Mühlau	Weir	Passable	2,84	2,84
	9	Salau	Weir	No Passable	5,24	4,33
	10	Engelsdorf	Rock ramp	Passable	7,61	4,11
	11	Weinburg	Weir	No Passable	1,71	1,71

This classification and the data about continuum interruptions were easily accessible. In fact, for the entire project background, most of the data were compiled in the report of Schöfbenker (2018). He realized the classification presented above, which will be used for the rest of interpretations. So, I need to find the right information needed for our project in the last report (Schöfbenker, 2018).

IV.2. Investigation conditions

The project studying the ecology of the *Hucho hucho* began on March 19th, 2023 with a spawning redds survey. The larval survey took place from May 9th, 2023 to June 14th, 2023. Between the 3rd of May and the 9th of May, I was looking for late spawning fishes and new spawning redds too. The study of available habitats took place from June 27th to June 29th, 2023.

As it was explained, the Danube life-cycle depends mostly on the water temperature and the discharge. The average water temperature was taken in three points along the Pielach: at the confluence with the Danube, at Wimpassing and at Hofstetten (the most upstream city in the study area). For the spawning, the best conditions were not reached at the same time between the downstream and upstream parts of the river. The lowest temperature is in Hofstetten and the highest in the confluence (Figure 4). During the spawning season, the temperature fluctuated between 5.3°C and 10.5°C in Hofstetten and between 6.8°C and 12°C in the confluence. The average daily discharge was around 15.4 m³/s but on the 15th of April, a big discharge took place. The maximum of this discharge reached 129 m³/s. The water level was too high to spawn and even after this flood event, no Danube salmon were found spawning anymore. The spawning season ended with this biennial discharge.

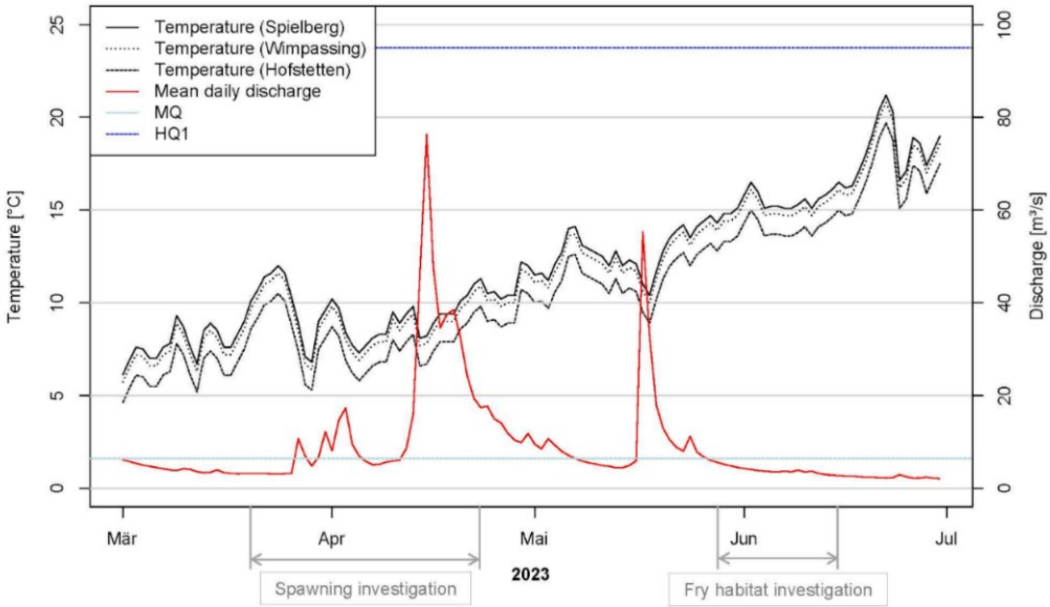

Figure 4 : Mean daily water temperature and mean daily discharge from the spawning season to the larvae mapping at Hofstetten, Wimpassing and the confluence. © Gabriel Gruber

Firstly, the larvae mapping was done from the shore, due to a high discharge preventing to go in the water, but with no relevant success. When the discharge was below 5 m³/s, it was possible to walk in the water. The average discharge during the larvae mapping was 4 m³/s (Figure 4). The average water temperature between the confluence and the upstream part of the study area fluctuates between 15.3°C and 13.7°C.

At the beginning, I needed to adapt a bit: I was in a new country and I had to go directly on the field. Moreover, it was a period of rush as we needed to find new fishes, spawning redds or larvae. Without new information, it would have been impossible to continue the project. That is why it was difficult to understand all the purpose and how to do the job: I was discovering the field, both in organisation and physical condition, I was starting to understand the main objectives of the project and learning to recognize Danube salmon larvae. The field work was particularly difficult with dense vegetation, the high level of water and bad weather conditions. My determination and motivation were the reason to perform on those days. Then, and thanks to my organisation skills, I was able to do my job with more confidence. Moreover, I was more at ease with walking in the water as I used to scuba dive. It is not the same experience but it helped me to understand quickly how to move in the river and where some larvae could hide.

IV.3. Spawning redd mapping

Spawning sites were identified and mapped by observing the river from the shore. Since salmon dig a redd, spawning redds are easy to spot from the riverbank (Figure 5). This methodology was also made to minimize disturbance for salmon reproduction. For each spawning redds found, the GPS coordinated and the distance of the spawning ground from the shore were noted, then the length and width of the spawning redds were estimated. When the salmon were still in the area, their size and sex were noted as well.

Figure 5: Danube salmon spawning redds in the Pielach to the left and cleaning of the substrate explained by hydrological conditions to the right © Boku university

During my research, I did not find any spawning redds. Sometimes, I thought that I found one but after verification, it was not a redd. In fact, it was difficult to distinct if the lighter substrate was a redd or just a consequence of the flow velocity. So, I learnt to observe the river: if the lighter substrate was higher than 1 m of long and very lanceolate, if the substrate was higher than 200 mm and if it was possible to explain the cleaning of the substrate by hydrological condition, it was probably not a redd. For example, on the right picture of figure 5, it is possible to see a suspicious redd but the substrate was higher than 200 mm and the position after the riffle could explain the deposit of clean substrate. So, it was not a redd but a formation created by hydrological conditions. The other difficulty was to look at the river all the time. Indeed, the riparian vegetation often prevented from checking along all the river. That is why the investigation area was prospected (Figure 3) several times to identify the formation of spawning redds throughout the spawning period by different people. A regular contact was also set up with local authorities to be noticed of spawning redds.

I didn’t find any redd during my spawning redd prospecting period.

IV.4. Habitat characterisation for *Hucho hucho* larvae

IV.4.1. Larvae mapping and used habitat characterisation

Larvae were surveyed by walking in the river. Wherever possible, both shores were prospected. The search of larvae could only be carried out when the flow was less than 5 m^3/s.

Of the 32 km surveyed, only 11.1 km were prospected for larvae because there wasn't enough time and resources to survey the entire study area. So, it was therefore decided to survey the 300 to 400 m downstream of the spawning grounds. The survey was always carried out from downstream to upstream. The oldest spawning redds were surveyed first. If larvae were found, they were captured, identified, measured and released. No larva was harmed during the process. The GPS coordinates of each habitat and its characteristics were noted.

To identify *Hucho hucho* larvae (Figure 6), several morphological characteristics were used: the presence of an adipose fin identified the Salmonidae family, the number of rays on the dorsal fin: 13 to 14 for Danube salmon, the position of the dorsal fin far back on the body.

Figure 6 : Photographs of Hucho hucho larvae. The larva on the right measures 26mm, while that on the left measures 37mm. © Emmanuelle Waguette and Illona Doyon

After the larva capture and release, the used habitats were characterized hydrologically by their size, the distance from the shore, the percentage composition of its substrate and its compactness, the flow velocity, the depth, the presence of xylal (structures) which can modify the initial habitat and the vegetation cover above and below 50 cm. The size of the habitat was defined by the area in which the larva was observed. Substrate compactness was defined empirically. The table 4 shows the different types and sizes of substrate. The flow velocity and the depth were measured at larva position, 50 cm from the bank, 1m and 1.5m were measured using the FLO-Mate (HACH) for surface velocity (V_{100}) and velocity at 40% depth (V_{40}). These two values are used to calculate the average flow velocity according to the equation: $V_{mean} = 0.31 \times V_{100} + 0.63 \times V_{40}$ (Kreps, 1975). The presence of xylal on the cover may play a role in protecting larvae from predators.

Table 4: Type and size of substrate

Substrate	Pelal	Psammal	Akal	Microlithal	Mesolithal	Macrolithal	Megalithal
Size	< 0.063 mm	0.063 – 2 mm	2 – 20 mm	20 – 63 mm	63 – 200 mm	200 – 400 mm	> 400 mm

IV.4.2. Available habitats

The difference with the habitats used by larvae is that the available habitats are present in the river but are not all used by Danube salmon larvae. To identify these habitats, two series of transects were carried out from the Pielach: one in the residual flow and the other in the full flow.

This decision was based on observations made during the larval survey. A difference appeared between the number of larvae present in the residual flow and in the full flow (these results will be presented later). It therefore appeared necessary to compare the available habitats in these two different flows.

To characterize available habitats, transects were spaced at around 40 m intervals at the start of a spawning area. A measuring tape was pulled across the entire width of the river. The points on the transect were spaced 2 m apart, except near the bank: the points on the bank level and the first on the river level were 50 cm apart. At each point, the hydrological characteristics of the surveyed habitats were similar to those studied for the used habitats: depth, vegetation cover above and below 50 cm, flow velocity (V_{100} and V_{40}), presence or absence of xylal, substrate composition and stability. While during the larvae mapping, the substrate stability was defined empirically, here it is studied by using a penetrometer.

The transects were carried out two weeks after the end of larval prospection, which implies differences in research conditions. In fact, the average flow was much lower (2 m^3/s) than during the larvae research. It was therefore difficult to make a direct comparison between the habitats used and those available, since the data were collected at very different flow rates.

However, this survey did enable us to define the available habitats for specific conditions and gave us an idea of the diversity of habitats in the Pielach. In this report, only a first analysis has been done as more details process would be used later.

IV.5. Data analysis

Raw data preparation was carried out by using Microsoft Excel (Version 2019). Mapping of spawning redds and larvae habitats was carried out by using QGIS (Version 3.28.2) and whisker boxes were made by using R software (Version 3.6.0). The other graphs were produced with Excel, like the Chi² tests. These tests were used to test the uniformity of spawning redds and larvae habitats between residual and full flows.

This part of the internship was really interesting. Indeed, I learnt to create some files that could be used and understood by other people. In fact, this data has been the basis of all interpretation I did and will still be used after the end of my internship. I used my knowledge about Excel and R software to create the raw data and I expended my skills about these softwares too.

V. Results and discussion

V.1. Results

V.1.1. Spawning redds mapping

During the spawning redd mapping, 58 spawning redds have been detected (Table 5) in the investigation area, which correspond to 1.78 redds per kilometre. The number of spawning redds is higher between the Danube and Salau (47 spawning redds) than between Salau and Weinburg (11 spawning redds). In the same way, the spawning redd density is higher in the first sub-population (2 redd/km) than in the second (1.2 redd/km). The highest density of spawning redd is between Doppel and Mühlau (7.2 redd/km) and the lowest is between Spielberg and Albrechtsberg (0.26 redd/km). No spawning redd was found in the sub-reach 3, 5 and 11.

Table 5: Number and density of spawning redds and larvae in the Pielach in 2023 in each sub-populations (ID : 1-2) and sub-reach (ID : 3-11). The density of the spawning redd is calculated on 32km of the study whereas the density of the larvae is calculated on the 11km of larvae prospection.

	ID	Continuum interruption	Number of Spawning redds	Density of Spawning redd (Redds/km)	Number of larvaes	Density of larvae (larvaes/km)
Sub-population	1	Salau	47	2,04	42	5,42
	2	Weinburg	11	1,16	47	14,03
Sub-reach	3	Spielberg	0	0	0	0
	4	Albrechtsberg	1	0,26	0	0
	5	Neuhofen	0	0	0	0
	6	Eibelsau	6	1,18	40	17,30
	7	Doppel	5	2,93	2	3,86
	8	Mühlau	17	7,20	0	0
	9	Salau	18	3,42	0	0
	10	Engelsdorf	11	1,43	47	14,03
	11	Weinburg	0	0,00	0	0
Total			58	1,78	89	8,02

In 2016, 11 more spawning redds were found than in 2023. In the sub-population of Salau, a similar number of spawning redds were detected: 43 in 2016 and 47 in 2023 (Figure 7). Half as many spawning redds were found in 2023 in the sub-population of Weinburg. In 2023, the spawning redds are more concentrated around the sub-reaches Mühlau and Salau whereas in 2016, the repartition between the sub-reaches seemed more regular along the study area. Some spawning redds were found in the sub-reaches Neuhofen and Weinburg in 2016 but none of them were found in 2023.

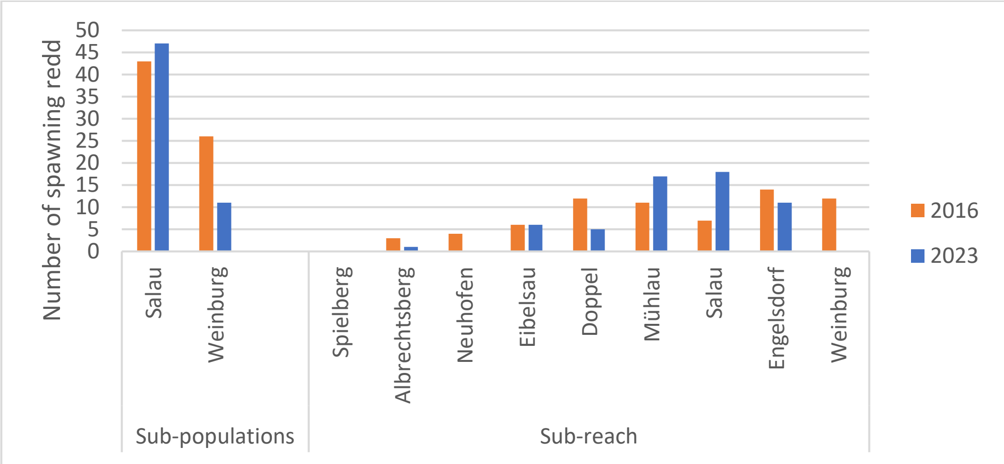

Figure 7: Number of spawning redds in the sub-populations and the sub-reaches in 2016 (Schöfbenker, 2018) and 2023.

The full flow of the Pielach stretches on 14.4 km whereas the residual flow stretches on 18.1 km. Most of the spawning redds are located between Doppel and Salau (which is confirmed by the spawning redd densities in the Table 5) and most of them are in the residual flow (Figure 8). Indeed, 18 spawning redds were found in the full flow, which correspond to 31% of the spawning and 1.25 redd/km. 69% of the spawning redds (40 spawning redds) were found in the residual flow. It corresponds to 2.2 redd/km. There is not any statistically difference between the density of spawning redds in the full flow and in the residual flow (p-value of the Chi^2 test = 0.609).

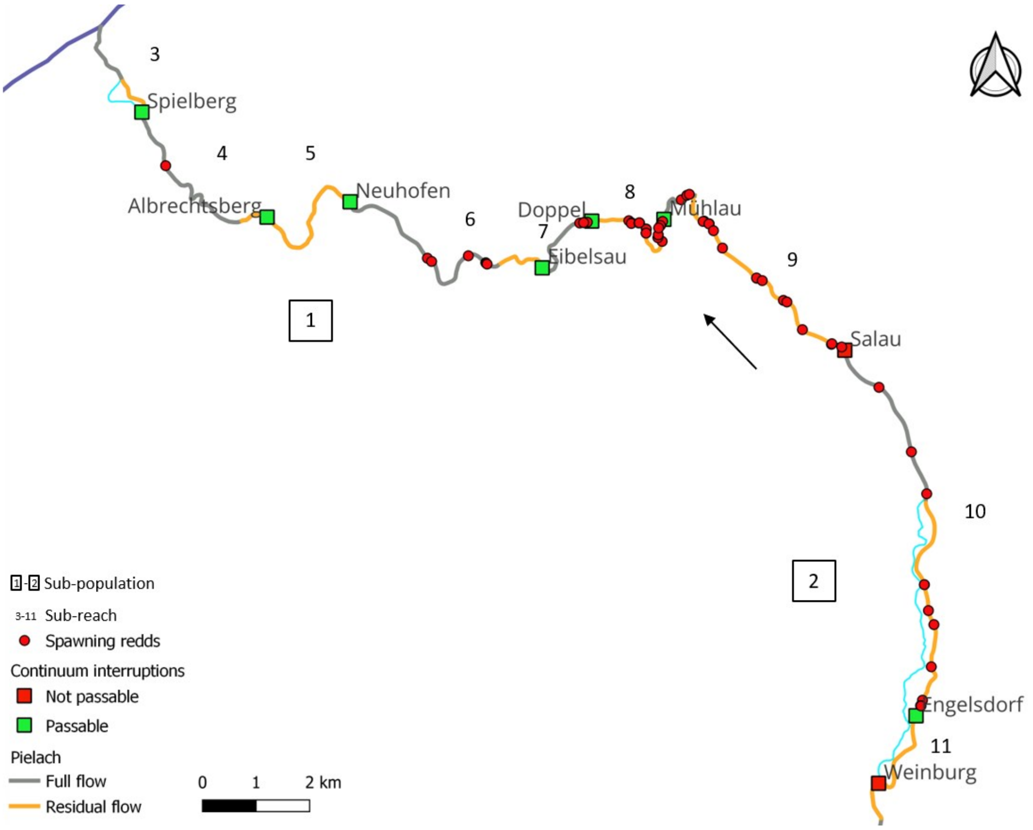

Figure 8: Detected Danube Salmon redds divided into two sub-populations and nine sub-reaches defined by continuum interruptions. © Emmanuelle Waguette & Illona Doyon

V.1.2. Larvae habitats

V.1.2.1. Larvae mapping

During the larvae mapping period, 89 larvae were found in 69 different habitats (Figure 9). The smallest larva found measured 23mm and the biggest 37mm, with an average of 29 mm for the larvae length. These sizes correspond to the early larval stages (from 20 mm to about 35 mm) of the Danube salmon (Pinter et al., in prep). So, it is possible to say that the larvae mapping corresponds to the first Danube salmon larvae habitats. As the larvae mapping lasted around 19 days, it was possible to observe a certain growth of the larvae.

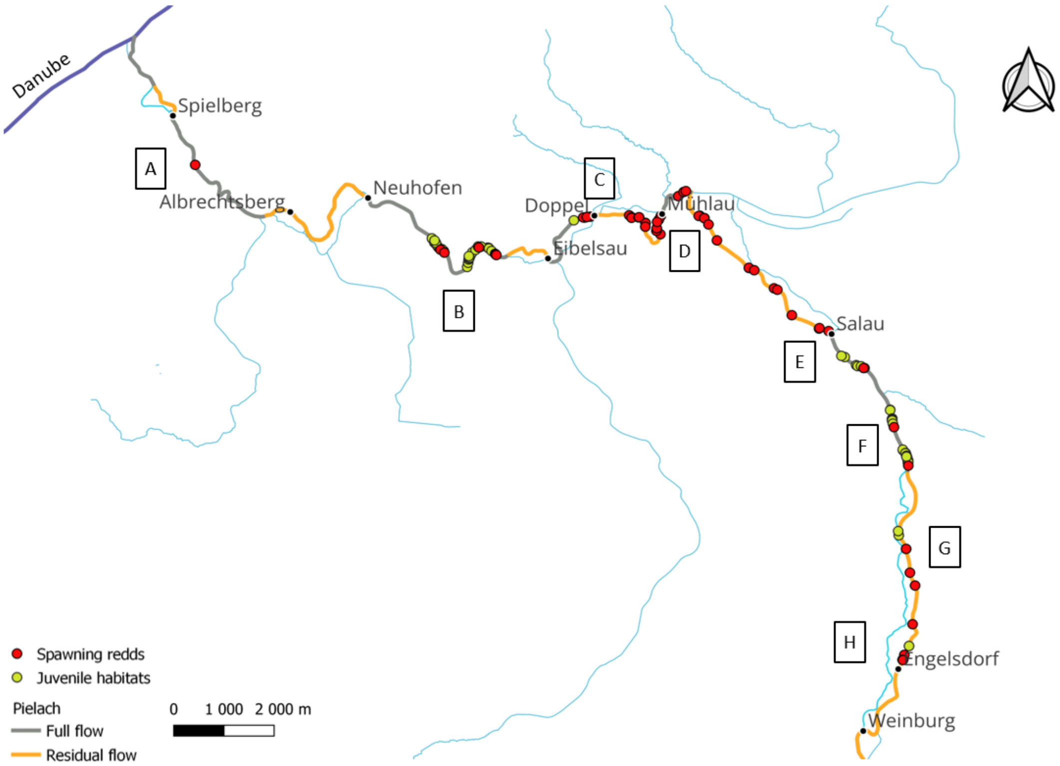

Figure 9 : Investigated Danube salmon juvenile habitats (n=69) from Weinburg to the Danube with both spawning redds and flow stretches. Boxes A to H are the zoom presented in the figure 11.

More than 50% of the larvae were located in the first 200 meters downstream of the spawning redds (Figure 10). The number of larvae seems to decrease when the distance from the spawning site increases. 95% of the larvae were found in the 400m downstream of the spawning redd. After 400m, just 2 or 1 larvae were found.

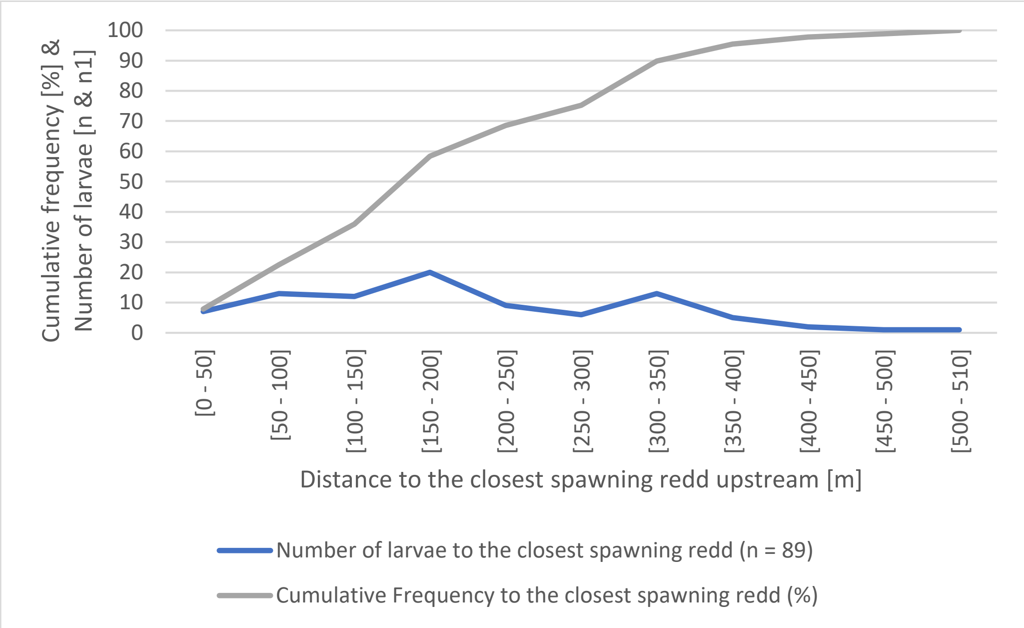

Figure 10: Distance of the fry habitats to the closest spawning redd upstream [m].

In the larvae prospected area, the density of the larvae represented around 8 larvae/km (Table 5). The sub-population of Weinburg (ID 2) welcomed a higher density of larvae than the sub-population of Salau: 14.03 larvae/km and 5.42 larvae/km respectively. This difference is related to the density of spawning redds. Indeed, almost half of the larvae were found in the second sub-population whereas only 19% of the spawning were located in this sub-population. Regarding to the sub-reaches, the larvae were concentrated in the sub-reaches of Eibelsau and Engeldorf: with 17.30 larvae/km and 14.03 larvae/km respectively. These two sub-reaches were in the full flow of the Pielach. For the sub-reach of Doppel, also in the full flow, only 2 larvae were found. In fact, a large majority of larvae were located in the full flow of the Pielach (Figure 11): only 6 larvae were found in the residual flow. In terms of density, 16.14 larvae/km were found in the full flow and 1 larvae/km were found in the residual flow. There is a statistical difference between the density of larvae in the full flow and the density of larvae in the residual flow (p-value $Chi^2 = 0.0003 < 0.05$).

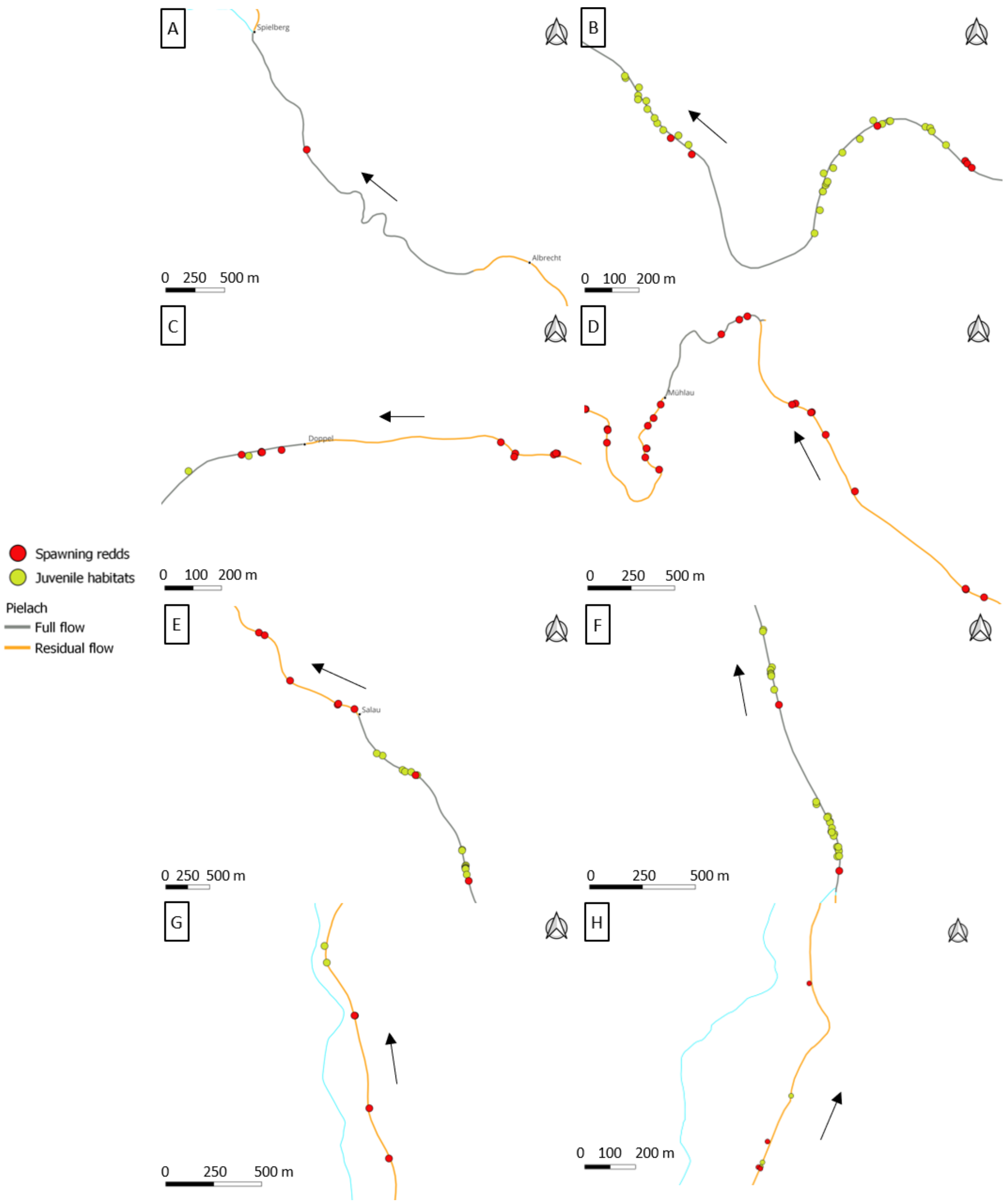

Figure 11: Detailed view of the larvae and redds mapping along the river course from downstream sites (A) to Weinburg (H). Arrow = flow direction © Emmanuelle Waguette & Illona Doyon

V.1.2.2. Used habitats

V.1.2.2.1. Different habitats

Used habitat show a lot of different appearances (Figure 12). All habitats show shallow water and flow velocity. Moreover, habitats d), e) and f) are characterized by coverage provided by riparian vegetation and dead woods. Habitat c) is an undercut bank. All this kind of habitats provide good protection for the larvae against predators. On the other hand, some habitats do not provide coverage but, for habitat a), stones could provide protection against high flow velocity, as same as overhanging vegetation. The last typical habitat (b) is characterized by shallow depth and low velocity but does not provide any protection from stone or vegetation.

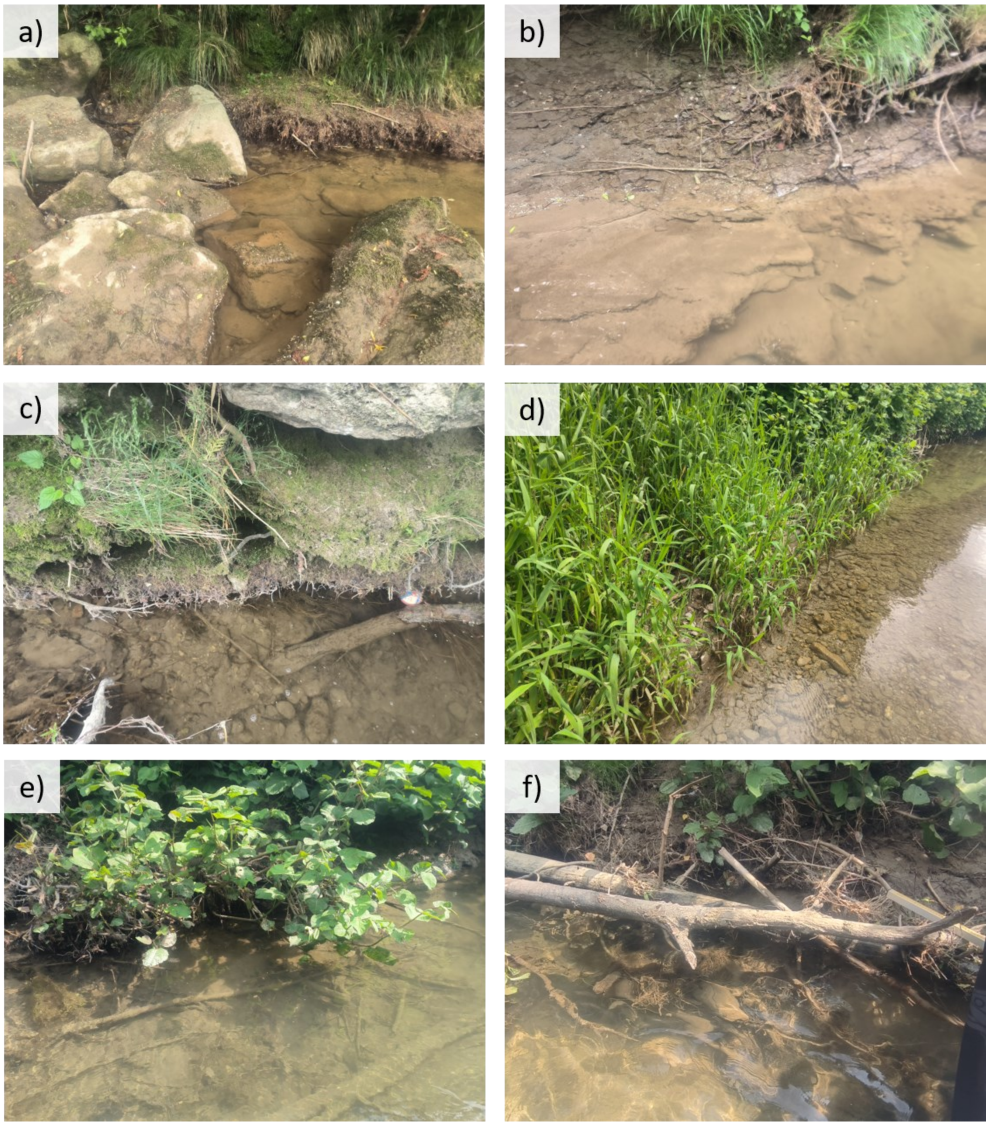

Figure 12: Examples of larvae habitats. © Emmanuelle Waguette & Illona Doyon

V.1.2.2.2. Used habitats characteristics

The average depth of all habitats is 23.3 cm, with a minimum of 6 cm and a maximum of 95 cm. The larvae were located at an average depth of 23.8 cm. Most of the larvae used a water depth between 11 and 21 cm (Figure 13), which represents 40.4% of the larvae. In fact, 75% of the larvae was located in water depth between 1 cm and 31 cm. Then, 90% of the larvae were in water depth below 51 cm.

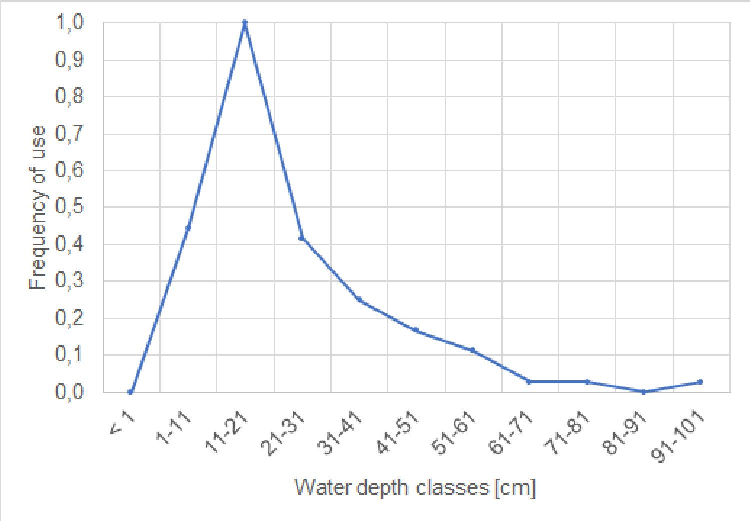

Figure 13: Frequency of depth water use by larvae

With the increase of the distance from the shore at the level of the larvae habitats, it is possible to observe an increase of the water depth (Figure 14), mostly between 50 cm and 1 m: the median is 25 cm at 50 cm from the shore and 40 cm at 1 m to 1.5 m from the shore. At 50 cm from the shore, 50% of the larvae habitat have a water depth between 15 cm and 35 cm and at 1 m from the shore, the water depth of the larvae habitat is between 35 cm and 60 cm.

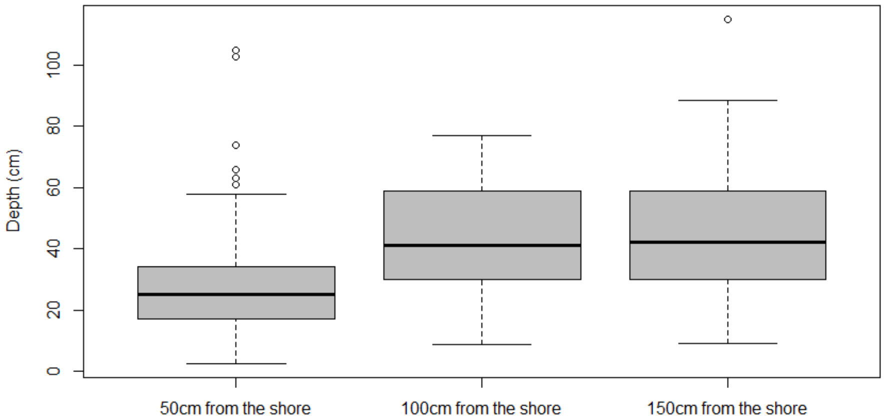

Figure 14: Boxplot of the water depth at 50 cm, 1m and 1.5m from the shore.

The frequency of use of the flow velocity at the fish position is very clear: the larvae are located in areas which present a flow velocity between -10 cm/s and 10 cm/s (Figure 15). Most of the larvae use a flow velocity between 0 and 10 cm/s.

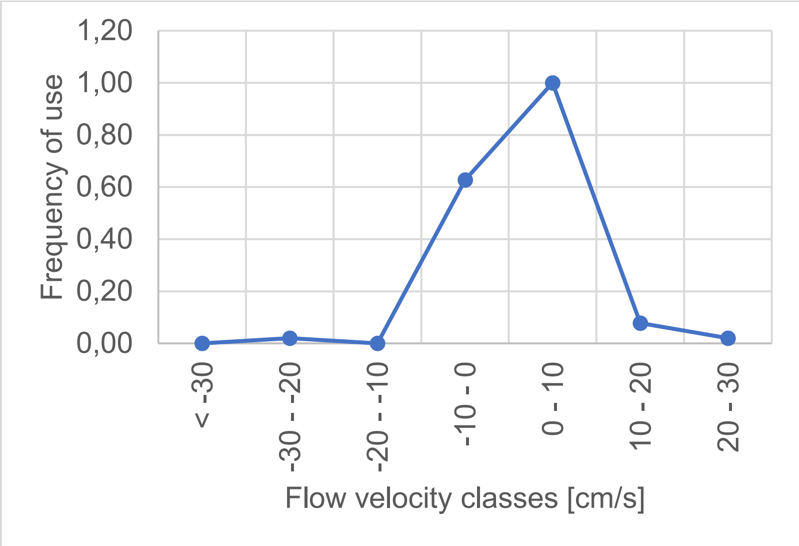

Figure 15: Frequency of use of the flow velocity (cm/s) by the larvae

At 50 cm from the shore, 50% of the average flow velocity is between 0 and 0.1 m/s whereas at 1 m from the shore, 50% is between 0 and 0.2 m/s (Figure 16). At 1.5 m from the shore, the average flow velocity is between 0.1 and 2.5 m/s for 50% of the value. It is also possible to see that the median is increasing with the distance from the shore: the median goes from 0.07 m/s to 0.2 m/s with the increase of the distance.

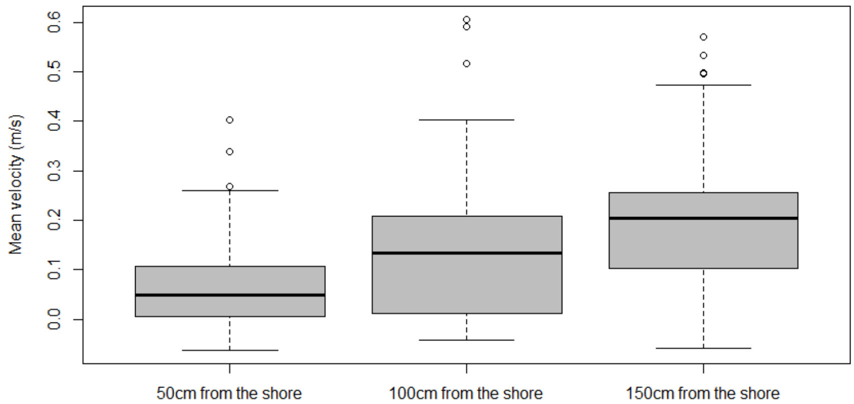

Figure 16: Boxplot of the mean flow velocity at 50 cm, 1m and 1.5m from the shore.

The Danube salmon larvae habitats are mostly composed of inorganic fraction: 45% of Pelal and 24% of Psammal (Figure 17). 13% of the habitat composition is represented by megalithal. Less than 12% is represented by mesolithal, microlithal and macrolithal.

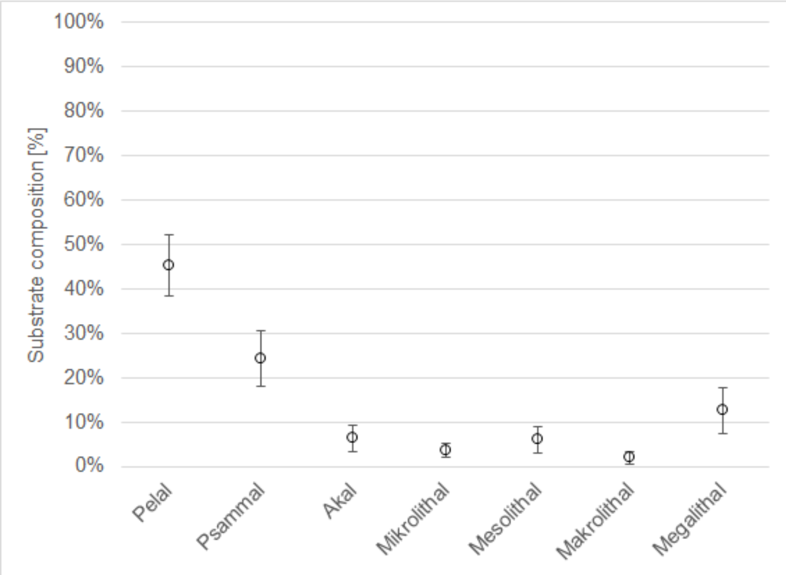

Figure 17: Dispersion of all sampled mean substrate types with the confidence interval at 95%.

The used cover was estimated below 50 cm and above 50 cm from the larvae habitat. Most of the cover type was made of riparian vegetation and stone. Xylal, like some wood in the water, could provide structure in the larvae habitats and some cover too. Most of the larvae habitats had xylal (81%). The average cover below 50 cm is 31% and above 50 cm, it is about 50%. Indeed, 50% of the larvae habitats had a cover below 50 cm between 15% and 50% (Figure 18). On the other hand, the coverage above 50 cm of 50% of the larvae habitats was between 30% and 70%.

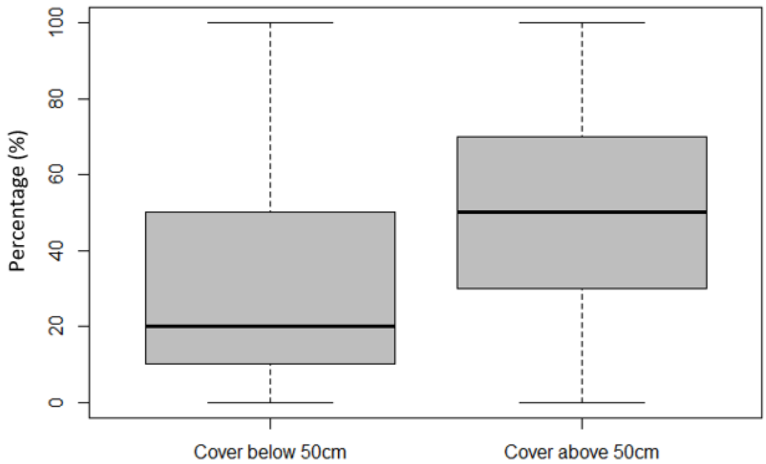

Figure 18: Boxplot of the coverage below and above 50 cm of the larvae habitats

V.1.3. Physical characteristics in full and residual flow habitats

V.1.3.1. Available habitats

It is important to remember that the analysis of the available habitats presented in this study shows only a first overview. Moreover, the data of used habitats and available habitats were recorded during different discharges. This comparison must be taken carefully knowing available habitats could be different with a higher discharge. As the data are not normally distributed, the median will be used to analyse the available habitats characteristics.

In the full flow and in the residual flow, the depth increases with the distance from the shore (Figure 19.A). Near the shore, the median of the depth in all habitats in the full flow is 11.5 cm and it is of 0 cm in the residual flow. At 40% of the distance from the shore, the median depth is about 40 cm in the full flow and in the residual flow.

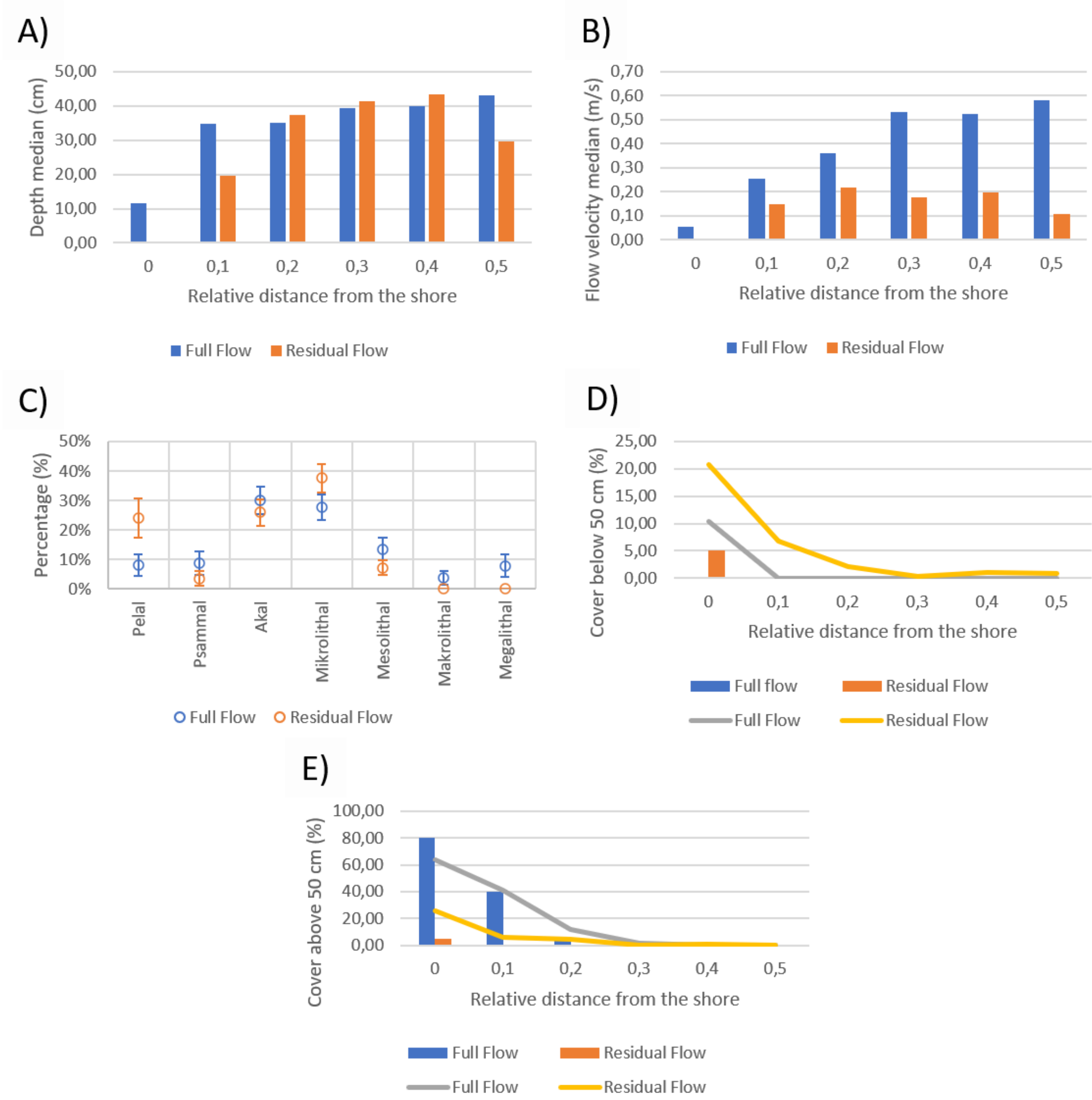

Figure 19: Physical characteristics of available habitats in the Pielach. Graph A presents the median depth (cm) depending on the relative distance from the shore. Graph B presents the median flow velocity (m/s) depending of the relative distance from the shore. Graph C presents the average of substrate composition with the 95% confidence intervals. Graph D and C present the cover below 50 cm and above 50 cm respectively depending on the relative distance from the shore. The bars are the median whereas the curves are average. For each graph, the full flow and the residual flow are analysed. The relative distance from the shore was calculated by dividing the distance from the closest shore by the total length of the transect.

As for the depth, the flow velocity increases with the distance from the shore (Figure 19.B). In the full flow, the median near the shore is 0,06 m/s and is 0.58 m/s in the middle of the river. In the residual flow, the median flow velocity near the shore is 0 m/s and fluctuates between 0.11 m/s and 0.22 m/s when the distance increases from the shore. Moreover, the flow velocity is almost three times smaller in the residual flow than in the full flow.

The residual flow substrate is composed of 38% of microlithal, 26% of akal and 24% of pelal (Figure 19.C). So, almost 90% of the substrate is composed of fractions below 6,3 cm. Neither macrolithal nor megalithal were found in the residual flow. In the full flow, all the fractions were found. The predominant fractions are akal (30%) and microlithal (28%). The fine fractions (pelal and psammal) are poorly represented (less than 20%), the macrolithal and megalithal are present (around 10%). The residual flow has more pelal than the full flow. It is due to the smaller flow velocity found in this part of the river. If fine fraction (<2 mm) represents almost a third of the substrate composition of available habitats in the residual flow, almost no larvae were found in this part of the river. On the opposite, most of the larvae were found in the full flow where the substrate composition of the available habitats was composed of less than 15% from fine fractions.

The average of cover, as the median, (below and above 50 cm) is decreasing with the increase of the distance from the shore (Figure 19.D and Figure 19.E). The coverage below 50 cm is more important in the residual flow whereas the coverage above 50 cm is higher in the full flow.

V.1.3.2. Substrate compactness

The compactness of the substrate in both full and residual flow was evaluated with a penetrometer device. It measures the maximum penetration of the device after some hitting. As the data were not normally distributed (Figure 190), it was not possible to use statistical method on the average. So, the median was used.

For the spawning redd and the transect evaluation, the maximum penetration in the substrate is higher in the residual flow than in the full flow: the median of the transect maximum penetration in the substrate is 23.3 cm for the residual flow and 10.1 cm for the full flow and it is 20.5 cm for the

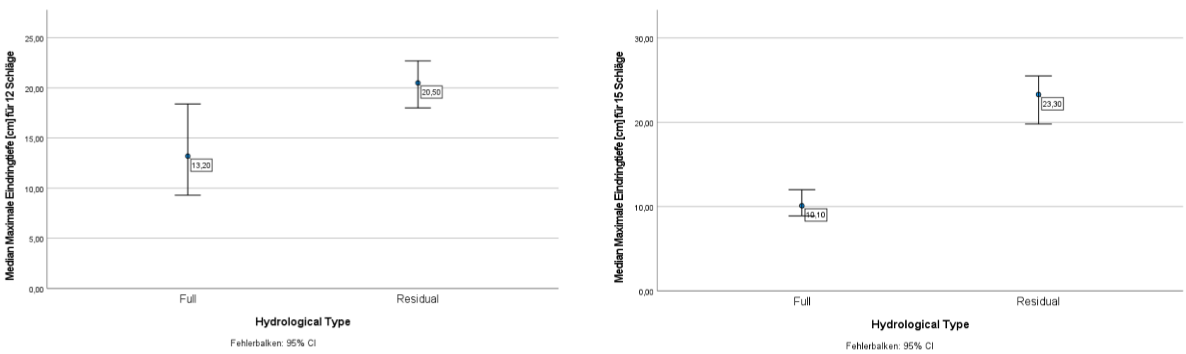

Figure 20: Maximum penetration depth (cm) in the full and residual flow for spawning redd (left graph) and transect (right graph)

spawning redds in the residual flow and 13.2 cm in the full flow (Figure 12). Moreover, there is a statistical difference for the transect between the residual flow and the full flow as the confidence intervals of 95% were not overlapped. For the spawning redd compactness, the confidence intervals between the full flow and the residual flow were a bit overlapping. It is possible to assume this overlap comes from the size of the sample. Indeed, the compactness data for redds is much smaller than the transect data (more than 200 data of difference).

V.2. Discussion

V.2.1. Danube salmon reproduction in the Pielach

Danube salmon could be found in all the Danube catchment (Pinter et al, in prep). Nevertheless, no spawning redds were found between the Danube confluence and Spielberg, it is possible to assume that no Danube salmon came from the Danube. Some individuals were seen from 2000 to 2003, in 2006 (Kaufmann, 2006) and in 2015 (seen by G. Unfer, Schöfbenker, 2018) but none in 2016 and in 2023 whereas local authorities also observed the Danube salmon spawning season in the Pielach river. So, no Danube salmon homing was seen in the last years. Moreover, the Danube salmon does not seem to have any preference between the residual flow and the full flow of the Pielach. Residual flows often provide reduced discharge which entails, in insufficient water depth, for redd digging (Pulg et al, 2013) and colmation of spawning ground (Sear, 1993; Sear et al, 2008; Obruca, 2015). In the Pielach residual flow, it seems to not be the case. The same observation was made in 2016 (Schöfbenker, 2018).

It was also surprising to find no spawning redd between Engelsdorf and Weinburg. In fact, even before the biennial flood, the discharge was higher than the discharge condition needed by the Danube salmon for its spawning. With this high discharge, Danube salmons stopped their spawn and time between the first high discharge and the biennial discharge was too short for the restart of the spawning. So, the spawning season stopped with the increase of the discharge and never restarted. This fact could explain why much less redds were found in 2023 than in 2016 in the upstream part of the Pielach. Stopping the Danube salmon reproduction season does not only impact the biennial flood. Indeed, a total loss of the eggs and first emerged larvae is expected after this exceptional flow event. But, with almost 90 larvae found, the reproduction of Danube salmon seems to be a success.

V.2.2. Danube salmon larvae habitats

Danube salmon larvae used habitats look very different from each other but they share similar characteristics. They are commonly located within a few hundred meters downstream the spawning site. Same observations were done in other studies (Siemens, 2017; Schöfbenker, 2018; Ratschan et al, 2021). This observation could be explained by the fact that the larvae swimming capacity is weak (Sagnes et al, 1997). Larvae use “active-passive” movement to reach appropriate habitats more downstream where they can develop (Pavlov, 1994; Braaten et al, 2011; Ihut et al, 2014; Lechner et al, 2016). Moreover, the predation rate increases if the larva goes further downstream than a few hundred meters (Lechner et al, 2016; Boys et al, 2016). That is why larvae used habitats are found near redds and why available habitats must be closed to the spawning redd (Schöfbenker, 2018).

Salmon larvae and juveniles of the genus *Hucho* prefer shallow water (below 50 cm) and low flow velocity (mostly below 20 cm/s) to live (Augustyn et al, 1998; Siemens & Schnell, 2017; Guttmann, 2006), whether in other rivers and species other than the Danube salmon. More specifically, Danube salmon prefer very shallow water, from 10 to 20 cm, and flow velocity between -10 cm/s and 10 cm/s, as it was shown by Schöfbenker (2018). Fine fractions (< 2 mm) are predominant in the substrate composition of larvae habitats, which fits with the low flow velocity recorded. Indeed, this type of fraction could only be deposed in areas with very low velocity (Cobb et al, 1992). In 2016, the same composition preference was observed. Moreover, this type of substrate was not predominant in the available habitat. The larvae habitats show a certain amount of coverage. The submerged vegetation and macrophytes were preferred by the larvae in 2016 (Schöfbenker, 2018). The coverage and the presence of xylal is important because it provides a shelter from predators and the water current (Ayllon et al, 2009).

So, used habitats found in 2016 and 2023 share very similar characteristics. Then, it is possible to validate HYP1. In fact, Schöfbenker demonstrated that Danube salmon larvae used habitats are similar to the others species of the family of salmonid (2018).

V.2.3. Impact of the residual flow on the reproduction success of Danube salmon

In 2016, larvae were found in both full flow and residual flow in 2016 (Schöfbenker, 2018), but it is not the case in 2023: larvae were only found in the full flow whereas some spawning redds were in these parts of the river. It could be that all spawning redds in the residual flow did not contain eggs. Indeed, females of the genus *Hucho* used to create multiple redds during one spawning season (Holčik et al, 1988; Fukushima, 1994). So, not every Danube salmon spawning redds contained eggs as the females check and choose the spawning redd habitats (Jungwirth et al, 2003; Holzer, 2011). If the physical parameters (flow velocity, substrate composition) do not correspond to the female demand, she abandons the area. As the environmental conditions were nearly the same in2016 and 2023, it seems hard to say that all the spawning redd in the residual flow were "fake". More probable hypothesis is that the biennial discharge destroyed the Danube salmon eggs in the residual flow.

Individuals of the genus *Hucho* cover their eggs with a substrate (Esteve et al, 2013). This coverage enables the eggs to have a certain protection against the environnemental condition changes and the predation. In the residual flow of the Pielach, almost a quarter of the substrate composition is pelal, which is more linked to the lower flow velocity than in the full flow. A substrate composed of less fine fractions is more stable and so, provides a better egg protection against very high flow velocity. So, the HYP2 could be accepted. For an endangered species as *Hucho hucho*, the loss of almost half of their redds during an exceptionnal flow event, which frequency will increase with climate change, is a big problem for the long-term viability of the population.

V.2.4. Methodological approach

The evaluation and the monitoring of fish populations are commonly done by counting the spawning redds (Beland, 1996, Thurow et al, 2012; Kovach et al, 2017; Butler et al, 2021). This method is easy to perform and non-invasive. This fact is important because the disturbance could reduce the reproduction success of the salmonid specie.

Nevertheless, it is important to know the limits of the method. Indeed, some of the spawning redds could be "fake". The Danube salmon female behaviour of creating some unused redds led to an overestimation of "true" spawning redds (redds with eggs). In 2000, Edo et al. estimated the percentage of "fake" spawning redds (redds without eggs) for the specie *Hucho perryi.* According to this publication, 20 to 30% of the spawning redds are fake. So, in 2023, between 40 and 46 redds could be "true" spawning redds. According to Holzer (2011), the size and the shape of the spawning redds are correlated to the eggs deposit. It could be interesting to study this fact for the *Hucho hucho* to improve the evaluation of the reproduction success.

Nowadays, the counting redd method is evolving from riverbank evaluation to imagery evaluation (Auerbach & Fremier, 2023). This new method, still in development, is more expensive but could provide better estimation of the physical parameters characterizing the spawning habitat and less observatory bias within the next years.

It is impossible to say if the capture and release of Danube salmon larvae during the investigation could impact their survival. During the studies, in 2016 and 2023, no fishes were harmed. But, after some weeks, larvae changed of habitats (Schöfbenker, 2018) and could not be found anymore.

V.2.5. Feedback

This project was very interesting as it enabled me to analyse my own data recorded in the field. Moreover, I discovered what "field work" meant: problem anticipation and adaptation, record of the data without mistake even if we were tired. We needed to be as rigorous as possible in the field in order to build up a reliable data set. The environment of work was wonderful. My tutors were available to answer my questions and issues and I was able to learn a lot near them. They enabled me to have an easy access to the data I needed.

However, I would have liked to be more integrated in the protocol conception. Indeed, it is easy to follow step by step a protocol but it is also important to know why what and for which analysis you are working. Moreover, as a future engineer, this skill would have been very interesting to develop.

VI. Conclusion

As one of the last viable Danube salmon populations, the Danube salmon population of the Pielach is very important for the specie *Hucho hucho* survival. The high number of spawning redd along the river show a reproductive activity of the population. Moreover, the Pielach offers a variety of habitats that enables, at all life-stages of the Danube salmon, to find one with their own preference. Danube salmon adult prefer run sections and water depth around 35 cm to spawn and create their redds whereas Danube salmon larvae prefer habitats with shallow water, low flow velocity with fine fraction in the substrate composition. Nevertheless, even if the Danube salmon used the residual flow in the same way as the full flow for their spawning and that the residual flow offers habitats matching with the preference of larvae, this part of the river is more impacted by the exceptional flow event. Indeed, the biennial discharge which took place in April 2023 destroyed almost all the spawning redds but those in the full flow have provided better eggs protection against the high flow velocity.

In a context of climate change and the decrease of the Pielach population demonstrated in 2018 (Schöfbenker), it appears that the restoration of the full flow along the river is a priority. The residual flow was created to provide hydroelectricity for the milk farm installed near the Pielach. However, with the improvement of the technology, the hydroelectricity dams in the Pielach could be replaced by one or two wind turbines (M. Mühlbauer, 2023, personal communication). This replacement could enable to restore the full flow of the Pielach. With that and other restorations as continuum ones, the viability of the Danube salmon population in the Pielach could be ensured on the long-term.

This internship experience enabled me to acquire and develop some skills. Indeed, I learnt how to recognize larvae fishes and so, I improved my knowledge and skills about fish identification. As a future engineer, I need to understand the data I should analyse. To do that, I need to perform field work. This internship enabled me to realise all the research process: from the data acquisition to their interpretation. So, I improved my data collect organisation skill. I used for the first time QGIS to perform spatial analysis. I am very happy that I could develop this skill. Moreover, I was integrated in a research team. This was very interesting as I needed to use my skills about communication and adaptation. Indeed, the work methods was a little bit different, as I needed to share my data with the team. So, my documents needed to be clear, readable in English. At the end, I worked in total autonomy. The team helped me at the beginning but after their explanations about the Danube salmon life cycle, the field work and data analysis, I performed all my tasks on my own.

These experiences and skill improvements will be useful for my future job. In fact, the use of English to communicate and the spatial analysis using are very important for the job I want. Moreover, I am now capable to organise and perform field work. My future professional objectives are to continue developing skills I have and acquired during this internship to be able to realise quality work as an engineer.

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Titre: Habitat assessment of juvenile Danube Salmon (Hucho hucho) in the River Pielach

Abstract: The Danube salmon (Hucho hucho, Linnaeus 1758), is endemic to the Danube basin. The Pielach river welcomes one of the last viable populations of this specie. However, this specie ecology is not well-known, especially during the first larvae life-stage. Spawning redds, larvae mapping and transects were realized to understand the Danube salmon used habitats and available habitats in the Pielach. This study enables to present the larvae habitat characteristics (shallow water, low flow velocity, substrate, etc…) and that the compactness of the substrate plays a part in the egg survival during exceptional flow event. In the climate change and biodiversity extinction context, the understanding of endangered species ecology like Danube salmon is important to enable the implementation of management and restoration solutions.

Keywords: Danube salmon, larvae, spawning redd, habitat

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