

Article

The Reintroduction of Brown Trout (*Salmo trutta fario*) in the Upper Scheldt River Basin (Flanders, Belgium): Success or Failure?

Pieter Boets ^{1,2,*} , Alain Dillen ³, Johan Auwerx ⁴ , Mechtild Zoeter Vanpoucke ¹, Wim Van Nieuwenhuyze ¹, Eddy Poelman ¹ and Peter Goethals ²

¹ Provincial Centre of Environmental Research, Godshuizenlaan 95, 9000 Ghent, Belgium;

mechtild.zoeter.vanpoucke@oost-vlaanderen.be (M.Z.V.);

wim.van.nieuwenhuyze@oost-vlaanderen.be (W.V.N.); eddy.poelman@oost-vlaanderen.be (E.P.)

² Department of Animal Sciences and Aquatic Ecology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium; peter.goethals@ugent.be

³ Agency for Nature and Forest, VAC Gent, Koningin Maria Hendrikaplein, 9000 Ghent, Belgium; alain.dillen@vlaanderen.be

⁴ Research Institute for Nature and Forest, Dwersbos 28, 1630 Linkebeek, Belgium; johan.auwerx@inbo.be

* Correspondence: pieter.boets@oost-vlaanderen.be

Abstract: In 2017, the reintroduction of juvenile brown trout in the southwestern part of Flanders (the Zwalm River basin) (Belgium) was initiated. Monitoring during the subsequent years indicated that the released juveniles survived and matured, indicating that sufficient food and good habitat conditions were available. Despite recent fulfilment of free fish migration within the Zwalm River basin and several spawning habitats being present, no natural reproduction of brown trout could be observed. To obtain more insight into the reproduction and maturing of brown trout eggs under natural conditions, an in situ experiment was conducted during 3 consecutive years at 10 different sites within the river basin. The results of our research indicated that egg survival was generally low (<5%). The main causes are most likely a heavy sediment load hampering sufficient oxygen and clean water flow through the redds. In this basin, the sediment load originates mainly from agricultural fields during heavy rain events and consequential run-off. Creating grassy and/or woody buffer strips along watercourses, in combination with changes in agricultural practices, is needed to be able to build up a viable and self-sustaining population of brown trout and also, in a larger context, of other rheophilic fish species.

Keywords: erosion; reproduction; brown trout eggs; Vibert box; water quality



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1. Introduction

The anthropogenic impact on the environment leads to a decline of global biodiversity. Freshwater ecosystems in particular show a high decline in biodiversity and related ecosystem services during the last decades [1]. The 2020 WWF Living Planet report indicates that freshwater fish populations across the world have declined by an average of 84% in the last 50 years [2]. This alarming trend is due to multiple pressures on freshwater ecosystems including climate change, habitat loss, overharvesting, alien invasive species introductions, intensive agriculture and water pollution [3]. Despite this overall negative trend, recent efforts have been made to restore freshwater habitats (e.g., [4–6]) and to set up large scale river restoration programs (e.g., MERLIN, <https://project-merlin.eu/>, accessed on 22 December 2023).

Flanders (Belgium) is characterized by a high human population density, intensive agriculture, highly fragmented nature, patchiness of land use and scattered housing [5]. Therefore, there are many challenges when it comes to protecting and restoring freshwater habitats and surface water quality of streams, rivers and ponds. During the 1980s the

water quality reached an absolute minimum in Flanders [5,7]. However, since the EU Water Framework Directive (early 2000s) was established the soil chemical water quality in Flanders has drastically improved, although no further improvement has been observed during the last decade [8,9]. Ongoing efforts have been made to restore freshwater habitats and consequently an increase in freshwater biodiversity has been observed, mainly in the priority areas set by the Flemish government [10]. During the past 20 years, measures have been taken including the removal of weirs or installing of fish passages, allowing free fish migration, and construction of sewer systems and wastewater treatment plants, leading to improved water quality. Following these actions, several species restoration programs have been set up to promote reintroduction of endangered species. Finally, large-scale re-meandering projects and construction of buffer basins and floodplains in the framework of the SIGMA plan (<https://www.sigmaplan.be/en/>, accessed on 15 September 2023) have contributed to hydromorphological restoration.

The Zwalm River basin, part of the upper Scheldt River basin, is an example of a river basin that has relatively pristine headwaters but shows high impact at the mid- and downstream sections [11]. During the last two decades, huge efforts have been made to restore these sections in terms of hydromorphology, water quality and free fish migration [5,12]. The Zwalm River basin has been well studied in the past (e.g., [5,13]) and several decision support models have been made to support river management and habitat restoration (e.g., [14,15]). This has contributed to several restoration actions taken by the water managers that have led to an improved habitat and water quality (see [5]). This also recently allowed conservationists to work on the reintroduction of rheophilic fish species including brown trout (*Salmo trutta fario*), a species with high habitat requirements [16]. A habitat suitability model for brown trout [17] showed that the Zwalm River basin had good potential for reintroduction of brown trout, and that the headwaters of the river basin are largely suitable. In 2017, the reintroduction started with juvenile brown trout bred at the Research Centre for Aquatic Fauna in Linkebeek (Belgium) using native breeding animals (the parent animals are descendants of an original native Belgian breeding stock without genetic pollution from neighboring countries [18]). The first fish stock assessments indicated that initial survival of juvenile brown trout was good and that, after one year, specimens up to a total length of 30 cm were caught [19]. At the same time that the introduction of brown trout started, existing spawning grounds were maintained, meaning that the gravel beds were raked before the spawning season. In addition, new spawning beds were installed, bringing coarse gravel substrate into the stream at sites where it was thought to be optimal (based on expert judgement). In the following years (2018, 2019, 2020), the Zwalm River basin was consecutively restocked with juvenile brown trout.

The reproductive cycle of brown trout consists of different stages. Adult brown trout spawn at the age of 2 to 3 years, after which the eggs develop into alevins. These alevins remain in the gravel and live on their yolk sac, after which they further evolve to fry. In the final step, the fry set up territories and grow into parr [20]. A review paper by Louhi et al. [21] indicated that the quality of the spawning beds is important for the survival of the eggs and fry and that juvenile fish have more restrictions in terms of habitat requirements. Depth, stream velocity and substrate grain size are generally considered the most important microhabitat variables determining the spawning habitat quality of stream fishes [22]. Furthermore, oxygen concentration and the amount of fine sediment in the substratum need to be taken into account when assessing the critical intragravel conditions for successful embryo development.

Assessment of the gravel beds present in the Zwalm River basin indicated that not all of them were suitable as spawning beds since some of them contained too much fine sediments, had too high stream velocities or were overgrown by algae [23]. Nevertheless, there were sites where the spawning beds were of good quality, theoretically allowing brown trout to spawn successfully.

Two and three years after the first reintroduction, several sites were checked for natural reproduction (before restocking) as a species restoration program can only be considered

successful if a self-maintaining population is present. Based on several assessments, no eggs or alevins could be found in the Zwalm River basin despite the habitat being suitable for the species to be able to reproduce (sufficiently high water quality, presence of spawning beds, etc.), with the exception of one smaller tributary in which trout parr were found using electrofishing techniques [19]. Therefore, an experiment was conducted using fertilized brown trout eggs to find out which factors are most decisive for reproduction success, allowing to make specific recommendations for optimization of the species restoration program. The specific aims of the study were: (1) to test the survival of brown trout eggs in the Zwalm River basin using Vibert boxes, (2) to check the relationship between the survival of the eggs and site-specific environmental conditions and (3) to provide recommendations to water managers to improve the potential reproduction of brown trout in the Zwalm River basin.

2. Materials and Methods

2.1. Study Area

The study took place in the Zwalm River basin, which is situated in the southwestern part of Flanders and is part of the Upper Scheldt River basin. The Zwalm River is 22 km long and consists of near pristine headwaters, but its downstream parts are severely anthropogenically affected and have a lower ecological water quality [14,24]). The physical habitat quality is still excellent in the forested upstream spring areas, but ranges from moderate to very poor in the middle and downstream sections of the river basin. The reason for this is the presence of scattered housing, flood control weirs, straightened river channels and artificial embankments at the downstream part of the river [11]. Although five wastewater treatment plants (WWTP) are operating in the basin and two more are planned in the next few years, at least 40% of the regional inhabitants still live in scattered population clusters which are not connected to the centralized sewer systems [8]. Therefore, the middle and downstream reaches still suffer from input of polluted water. There is free fish migration from the River Scheldt all the way up to the headwaters of the River Zwalm. Given the presence of rare and protected species in Flanders [25] such as brook lamprey (*Lampetra planeri*, IUCN red list: least concern) and brook bullhead (*Cottus rhenanus*, IUCN red list: least concern), the river basin is set as a priority area for achieving good ecological status by 2027 [15].

The topography of the basin is best described as rolling hills, mild slopes and altitudinal differences of up to 150 m. The soil of the land consists of sandy loam. The land use within the basin is mainly agriculture (arable crops and pasture) with about 10% urban land cover. Soil erosion is one of the most important challenges in the basin, which results in considerable transport of sediments throughout the river system [13,26].

The average annual rainfall in the Zwalm River basin is 832.9 mm [27]. Due to the topography, the river basin quickly reacts to rainfall and an almost immediate run-off of sediment towards the river has been observed [28]. Measurements at several sites within the Zwalm River basin indicated that the size of the dominant sediment fraction of total suspended solids in the river was 25–30 µm. The average annual sediment load towards the Zwalm River basin was estimated, based on a combination of measurements and models, at 1.5 ton/ha [26].

For more details on the study area, see Boets et al. [5], Boets et al. [17] and Forio et al. [13].

2.2. Methodology

We conducted in situ experiments with Vibert boxes in 3 consecutive years (2019–2020, 2020–2021 and 2021–2022) at a total of 10 different sites scattered throughout the Zwalm River basin (Figure 1 and Table S1). At each test site, three replicate Vibert boxes were placed, filled with 200 freshly fertilized eggs each. The experiment was not run at all 10 sites each year because of knowledge gained during the previous year (e.g., location not suitable) or because of practical considerations (accessibility, construction works planned, etc.). An overview of the experiment details can be found in Table S1. The experiment was carried

out using biodegradable plastic boxes (7 cm × 4 cm × 3 cm, C.O.F.A.), also known as Vibert boxes, with small mesh size openings that allow juveniles to escape after hatching. Each box was filled with exactly 200 fertilized brown trout eggs obtained from the Research Centre for Aquatic Fauna in Linkebeek and originated from a mix of different parental native animals. Within 12 h after egg fertilization, the boxes with the eggs were transferred to the selected sites in a cooling box. Per site, three Vibert boxes (replicates) were placed in a squared box (20 cm × 20 cm × 25 cm, mesh size 1 cm²) consisting of metal mesh and were filled with coarse gravel substrate (16–32 mm). These boxes were placed in the gravel bed at about half of their depth (10 cm). The boxes were placed in the watercourse in such a way that they had a constant flow of fresh water, but in such a way that the eggs could not move which is important for normal embryo development.

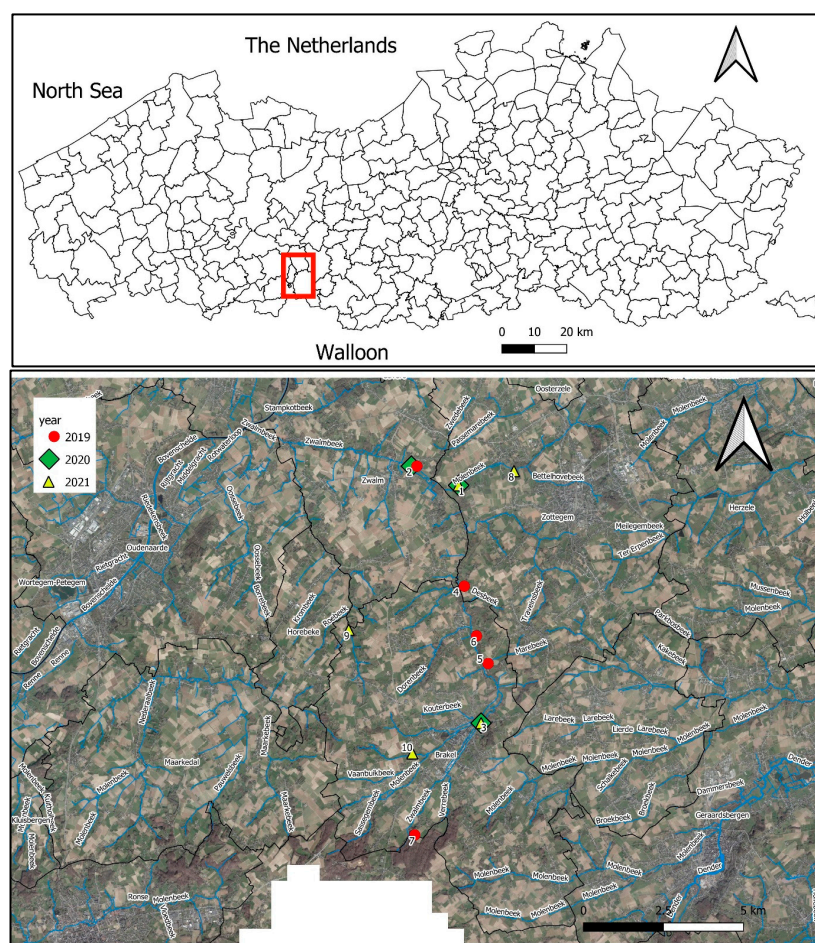


Figure 1. (Top): geographic situation of the Zwalm River basin within Flanders (see red rectangle). (Bottom): Overview of the watercourses (blue lines) and the sites where the experiment took place in the Zwalm River basin. Different colors and symbols are used for different years.

In the first year (2019/2020), the experiment started on 17 December 2019 and ended on 5 February 2020. The second year the experiment started on 18 December 2020 and for the third year on 17 December 2021. The end dates for the second and the third year were 16 February 2021 and 2022, respectively. The end date and thus the final control was set based on the time needed for the eggs to develop. The date was chosen as close as possible to the hatching date of the eggs and was calculated based on the water temperature. From previous research [29], it is known that brown trout needs about 400–460 degree days before the embryos hatch. The degree days are the sum of the average daily temperatures. In cooler water or harsher winters, it takes longer for the embryos to develop. This is why there were different end dates in the current experiment. Measures of water

temperature were obtained from the online web application of the Flemish Environment Agency (<https://www.vmm.be/data/waterkwaliteit>, accessed on 5 December 2023).

A control group of three Vibert boxes, also filled with 200 fertilized eggs each, were kept in the Research Centre for Aquatic Fauna in Linkebeek. These control boxes were kept under optimal conditions (sufficient flow and aeration, no sedimentation) at an average temperature of 10 ± 1 °C. This allowed comparison between the survival and developmental progress of eggs in situ and eggs kept under reference conditions.

At the end of the experiment, the number of eggs that survived were counted. Eggs were considered alive if embryonic development could be noticed (e.g., black eyes) or when the eggs were transparent with a typical pinkish color [30]. Eggs that turned opaque (whether white, brown or black) or showed signs of fungus were considered dead or not successful [31]. The survival rate (expressed as percentage) was calculated as the number of eggs that survived divided by the initial number of eggs (200) and multiplied with 100. The number of eggs that survived in the field was corrected for mortality in the control/reference set up.

To assess the influence of chemical and physical habitat conditions on egg survival, data retrieved from the Flemish Environment Agency monitoring network was used: suspended solids (mg/L), oxygen concentration (%), temperature (°C), pH, conductivity ($\mu\text{S}/\text{cm}$), basic Prati index and oxygen Prati index. The basic Prati index is calculated based on the values of chemical oxygen demand, oxygen saturation and ammonium nitrogen. The oxygen Prati index is calculated based on the oxygen saturation. These indices represent the chemical water quality status [32]. The variables were represented using one average calculated value (based on the measurements over one year preceding the experiment (Table S2)). The effect of erosion was assessed using maps of the erosion potential, available as a layer in GIS (see Figure S1). The maps indicate the erosion potential on a scale from 0 to 5, where 0 indicates no erosion and 5 indicates very high erosion potential. Within a buffer of 500 m around the experiment site, the erosion potential was calculated in GIS. The erosion class that dominated within this 500 m radius was linked to the study site. In our study sites, only the classes very low, low, medium and high occurred. The erosion classes were used to assess the relationship between the survival of the eggs and the erosion potential.

Next to the above analyses and calculations, visual observations were also made in the field each time the survival of the eggs was checked, and pictures were taken at each study site to document the state of the eggs.

2.3. Data Analysis

All statistical analyses were carried out in R software (version 3.6.2). All maps were made in QGIS software (version 3.10.9). The survival of trout eggs at the different sites was summarized in Table 1. The relationship between environmental and habitat conditions and the survival of eggs was tested using linear regression models and visualized using box plots or dot plots. Differences between years and between sites were tested using a Kruskal-Wallis *H* test (pgirmess package in R version 4.3.2 [33]), followed by a post-hoc Wilcoxon's test. If the *p*-value was lower than 0.05, differences were considered significant.

Table 1. Average and standard deviation (Stdev) of brown trout egg survival (%) based on the three replicates per site (and the reference site) and per year. Additional information on the sites can be found in Table S1.

Site	Year	Average Survival (%)	Stdev
2	2018–2019	10.5	8.8
4	2018–2019	1.0	0.9
5	2018–2019	0.0	0.0
6	2018–2019	0.0	0.0
7	2018–2019	12.2	4.9

Table 1. Cont.

Site	Year	Average Survival (%)	Stdev
Reference	2018–2019	98.0	2.0
1	2019–2020	2.0	3.1
2	2019–2020	5.1	7.9
3	2019–2020	6.8	11.7
Reference	2019–2020	76.0	3.0
1	2020–2021	0.0	0.0
3	2020–2021	70.2	10.9
8	2020–2021	2.5	1.3
9	2020–2021	0.0	0.0
10	2020–2021	0.0	0.0
Reference	2020–2021	49.8	38.8

3. Results

The overall survival of brown trout eggs in in situ experiments was relatively low (Table 1). At several sites there was no egg survival. The highest survival was observed at location 3 (town center of Brakel) with an average egg survival of $70 \pm 10\%$ in 2021. No significant differences in survival were found between years ($p > 0.05$), but significant differences were found between sites ($p < 0.05$). More upstream and thus more pristine sites did not have a higher egg survival compared to downstream sites. The survival of the reference kept in Linkebeek was high in 2019 and 2020 (between $76 \pm 3\%$ and $98 \pm 2\%$), though not in 2021 where it was lower ($50 \pm 40\%$ survival), although the differences between years was not significant ($p > 0.05$).

At the site with a high erosion potential, the egg survival was significantly lower ($p < 0.05$) compared to the sites with a very low erosion potential (Figure 2 and Table S3). Conductivity showed a significant ($p < 0.05$) negative correlation with egg survival (Figure 2). For the basic Prati index, the sites with the highest index (average and good), and thus a relatively good chemical water quality, also scored best in terms of egg survival, although the differences were not significant ($p > 0.05$). Based on the linear regression model we saw when including all available variables, the basic Prati index, conductivity and erosion potential significantly contributed to explaining the survival of the eggs ($p < 0.05$) (Table S4).

Visual observations in the field indicated that sedimentation within the gravel as well as in the Vibert boxes was high (Figure 3), and that during the experiment the eggs got clogged with fine sediments hampering the free flow of oxygen-rich water.

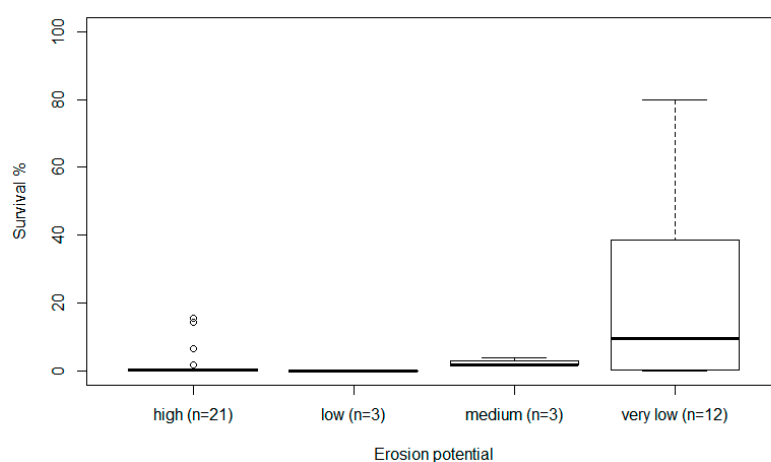


Figure 2. Cont.

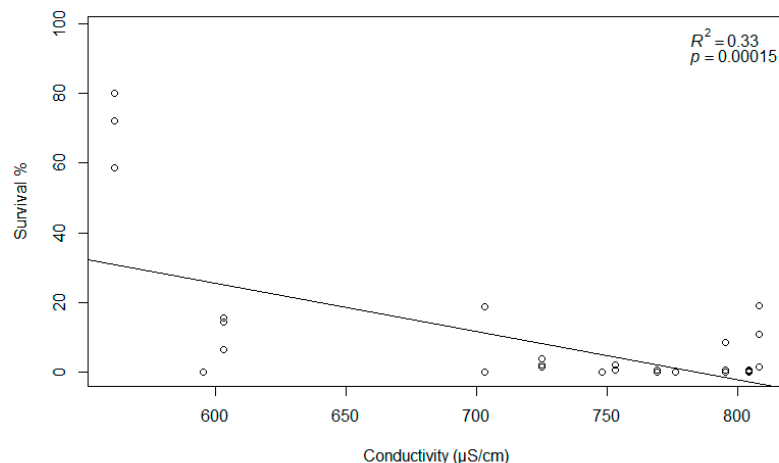


Figure 2. Brown trout egg survival (%) at each erosion potential level (**top**) and relationship between conductivity and brown trout egg survival in the experiments conducted in the Zwalm River basin in 2019, 2020 and 2021 (**bottom**). The solid black line indicates the fitted regression line.



Figure 3. Metal boxes (20 cm × 20 cm × 25 cm) filled with coarse gravel and clear indication of a high sediment load (**left**) and Vibert boxes (7 cm × 4 cm × 3 cm) with mainly dead eggs (**top right**) and eggs fully covered with sediment (**bottom right**).

4. Discussion

Based on our in situ experiments, it can be concluded that the survival of the brown trout eggs in the Zwalm River basin is rather low. Under natural conditions, mortality of the eggs of brown trout is very variable and earlier research has shown that survival can vary between 0.3 to 98% [20,34]. Several aspects affect the survival of the eggs, such as water quality, total suspended solids, presence of predators, resident populations of brown trout, the geographic location within the river basin, etc. [35,36]. The site-specific conditions and the interaction between different environmental and biotic conditions make it difficult to compare results of survival between studies and even between sites or geographic regions. However, our visual observations in the field (Figure 3), the data on water quality measurements and the results of the survival experiments with Vibert boxes strongly support the findings that egg survival in the Zwalm River basin is low. This can possibly limit natural reproduction success. The results of the in situ egg experiments are strengthened by fish research that took place in the Zwalm River basin after the introduction of juvenile trout. The survival of reintroduced juvenile trout (5–8 cm) was good, but young-

of-the-year trout (natural reproduction) were not found when carrying out fish research [19]. There is one exception within the basin where a relict population of brown trout has persisted in one smaller tributary of the River Zwalm for more than one decade [37].

The low survival of the eggs is thought to be mainly attributed to the high sediment load present in the river, caused by erosion due to run-off from agricultural fields. This phenomenon typically occurs in the region after heavy rain events taking place from December to February. The region is known for its high erosion potential hampering ecological restoration [5,26,38]. Spawning habitats of brown trout and rheophilic fish species in general seem to be very vulnerable to sediment accumulation [39]. When sediments cover brown trout eggs, the flow of fresh water and thus sufficient oxygen is hampered which leads to the suffocating of the embryos [40]. Conallin [41] found in Danish rivers that, due to human activities (mainly agriculture), the sediment load was well above the hatching–emergence critical limit, which contributed to minimal survival in the studied streams. In addition, an inverse relationship between quantity of fine sediment, egg survival and fry emergence has been demonstrated on all continents containing brown trout [42]. Our study results show that at sites surrounded by agricultural fields with a high erosion potential the survival was low, whereas at sites with low erosion potential the survival was significantly higher.

Based on a meta-analysis of brown trout egg development and reproduction, it was found that trout redds were mainly located in depths of 15–45 cm, that the preferred stream velocity was 20–55 cm s^{−1} and that the grain size of the substrate was between 16 and 64 mm in diameter [21]. In addition, it was found that oxygen concentration is preferably above 7 mg/L and that the percentage of fine sediments present in the spawning beds should be as low as possible. Michel et al. [36] found that not only the oxygen level itself but also the duration of low oxygen levels and the timing in the hatching process influenced the egg development. A higher percentage of fine sediments (<2 mm) showed a negative effect on egg survival since it reduced the availability of oxygen in the redds [41]. However, research specifically focusing on the relationship between fine sediments and the survival of salmonid eggs has not always shown straightforward results (e.g., [43]). Alberto et al. [44] found that temperature rather than sediment was the key variable in brook trout egg/embryo survival. In the latter study, trout specifically searched for breeding sites where upwelling groundwater was present to avoid clogging of the sediment and thus providing sufficient oxygen to the redds. Similar results related to water quality and fine sediment presence were reported by Lukenbach et al. [45] while studying the embryonic development of brown trout eggs at two different streams in Germany, characterized by different levels of pollution and human impact. Finally, Michel et al. [36] found no significant effect regarding the amount of fine sediment, but indicated that redd gravel permeability, which was affected by fine sediment deposition, was important for egg development.

Although most of the prerequisites in terms of environmental conditions found in the literature for brown trout egg development are met in our study area, some conditions seem currently inadequate for brown trout egg development in the Zwalm River basin. In particular, the limited presence of suitable spawning substrate and the high sediment load during heavy rain showers, in combination with low water quality during rain events, are the main bottleneck hampering reproduction of the species. In addition, changing environmental conditions due to climate change, such as longer droughts and thus lower water levels and reduced flow velocities, are observed in the Zwalm River basin (<https://www.waterinfo.be>). Climate change has shown to cause higher water temperatures, but also longer dry periods followed by intensive rain events, causing storm sewage at wastewater treatment plants. This leads to extra pressures on water quality and thus the survival of trout eggs [46].

In terms of suitable spawning habitat (grain size of substrate) within the Zwalm River basin, water managers have for years put serious efforts into providing new spawning beds on sites that were indicated based on hydromorphological and physico-chemical

research to be suitable. In addition, in cooperation with local volunteers, the spawning beds are raked every winter just before spawning of rheophilic species. Research in Southern Germany indicated that both gravel addition and gravel cleaning supported the creation of spawning grounds for brown trout [47]. In the first 2 years after restoration, highly suitable conditions were maintained, with a potential egg survival of more than 50%. Afterwards, the sites offered moderate conditions, resulting in an egg survival rate of less than 50%. After 5 to 6 years conditions for reproduction became unsuitable.

For brown trout, which seem to be more vulnerable compared to chub or dace to habitat degradation, extra measures and continued efforts are needed [21,47]. Recently, several national (e.g., Water + Land + Schap) and European funding projects have been started (e.g., MERLIN) to install buffer strips along watercourses to reduce erosion and improve the chemical water quality. Currently, the development of buffer strips next to watercourses is mostly still on a voluntary basis. Only on those fields categorized as “a very high erosion potential” are measures mandatory [48]. A modeling study by Borsuk et al. [49] in Switzerland on the survival of brown trout has indicated that if certain human pressures (in this case erosion and thus clogging of the spawning substrate) are reduced, the survival of brown trout can be tremendously increased. This supports the need for a solid legislative framework providing clear long-term goals and a solid and sustainable financial model for the farmers. Since diffuse pollution and erosion are by far the most important elements affecting the survival of sensitive aquatic species [50], farmers are the key stakeholders to communicate with. A solid agreement and long-term perspective for the farmers would help the realization of buffer strips on a permanent and sustainable basis. Upcoming legislation with regard to N emissions and the new manure action plan 7 (MAP 7, <https://www.vlm.be/nl/nieuws/Pages/MAP-7.aspx>, accessed on 5 December 2023) could probably have a positive effect on the permanent development of buffer strips next to watercourses. In addition, there are plans to reconnect a small tributary (Boembeke) which maintains a low sediment load even during heavy rain showers (pers. comm. Natuurpunt Zwalmvallei). These restoration measures could add substantial spawning and nursing habitat for brown trout. The outcomes of this research need to be further combined with other key species as well as objectives of local and international water governance methods in a context of sustainable development [51].

5. Conclusions

The survival of brown trout eggs in experimental Vibert boxes was generally low at most experimental sites in the Zwalm River basin. The average water quality and high sediment load due to erosion probably has a negative effect on the development of the eggs due to the lack of free-flowing oxygen rich water through the redds. There is currently only natural reproduction of brown trout observed in the Zwalm River basin in one small upstream tributary. The implementation of nature-based solutions such as buffer strips next to the watercourse are highly recommended to reduce diffuse pollution and increase the possible success of brown trout reproduction.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w16040533/s1>, Figure S1: Location of the experimental sites and the erosion potential of the surrounding areas; Table S1: Overview of the different sites where the in situ experiments took place in the different years; Table S2: Average values of the different environmental and biotic variables that were linked to the specific research sites; Table S3: Detailed statistics of the post-hoc multiple comparisons based on Wilcoxon’s test.

Author Contributions: P.B. and J.A. designed the study. P.B. led the writing. M.Z.V. and W.V.N. helped with the field experiments and data analyses. E.P., A.D. and P.G. assisted with the writing and gave comments on earlier drafts. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Raw data on water quality measurements that were analyzed in this study can be retrieved from <http://geoloket.vmm.be/Geoviews/> (accessed on 20 January 2024).

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