Entry to the Stockholm Junior Water Prize 2023:

Examining Sediment Quality and its Influence on Brown Trout Egg Development with an Innovative Sediment Trap

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I. Abstract

What is known as the aftermath of intensified flood events has similar impact on life below water. This increases the entry of fine sediment particles into streams as well as periodically dried soil due to a higher erosion and sediment mobility, combined with extensive agricultural land use along the water edges. This endangers key-stone species especially the brown trout *salmo trutta fario* due to clogged gravel-gap-systems they need to spawn. Within this study, a sediment trap was developed as a method for long-term sampling during spawning time and tested in five Bavarian streams. Furthermore, the impact of these sediment compositions on the development of brown trout eggs was determined to improve the success of in-situ-floating incubators to restore their populations.

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IV. Table of abbreviations and acronyms

TUM - Technische Universität München, FÖJ - Freiwilliges Ökologisches Jahr, e.V. - eingetragener Verein (registered association), Fig. - figure, ST - Sediment Trap, HEST - HydroEco-Sedimentary Tool, SP - Sampling Point, EOS - eyed ova stage, AWI - Alfred Wegener Institut, E.g. - example given, ISFIC - in-situ-floating incubator campaigns, LDPE pipettes - low density polyethylen pipettes, ANOVA - Analysis of variance, LfU - Landesamt für Umwelt

V. Motivation

Water has always been my element. Or rather my molecule. Growing up right beside the sea in summer and near Augsburg whose water management system has been declared an UNESCO World Heritage, I deeply appreciate the vital importance of water. As a Girl Scout since the first grade of elementary school it has been my aim to pass this appreciation and the knowledge, going hand in hand with it on to other people. This is why I write articles related to sustainability, science and water for the "Augsburger Allgemeine" and the "Nordsee Zeitung".

Moreover, I have been fighting against the entry of microplastics into the environment for 5 years now with the project "STOP! Microplastic". The project started in 2018 with the development of a microplastic filter for washing machine, advanced with an electronic alarm system and lateron resulted in the adaptation of the system for the laundry dryer. On that journey, the project was awarded with e.g. the Deutscher Engagementpreis 2019, silver at the iENA (internationale Erfindermesse Nürnberg), the Young Science Kika Award 2020, the finals of the GREENTEC Award 2019, nomination for the Audi Generation Award 2021, the Sustainability Award of the University of Ingolstadt, the Swabian Environment Award, the Future Award of Augsburg, the representaion of Jugend forscht at the Week of Environment in Berlin 2021 and various prizes at Jugend forscht. The network I built at all these unique opportunities is something that is very dear to me. It also gave me the opportunity to hold a speech at the World Scout Jamboree 2019, Fridays for Future and Podium Discussions. Together with my younger sister Zoë, I started the "Müllschmelze" (= trash melt). We collaborated with students from all over Germany to collect trash alongside rivers and streams to prevent the snow melt from washing it in. These flowing waters hooked my interest in them even more so that I started this very project. Originally, it was my graduation thesis but I wanted to contiue. That is why applied for the FÖJ, voluntary year at the department of Aquaculture Research at the Alfred-Wegener-Institut in Bremerhaven. Since August 2022, I have been working there now and continued this project after gaining some insight. Initiating a collaboration with instutions such as the Wasserwirtschaftsamt or the scientific water service really motivated me to expand this to more fishing associations.

1. Introduction

What is known as the aftermath of recently increasing flood events has had all the more dramatic cosequences on life below the water surface. One species that has been affected for example is the brown trout *salmo trutta fario* as a key-stone species in European streams and rivers. The input of fine sediments, < 2 mm sized particles [1] into the latter increases, due to anthropogenic influences as well as climate change. The intensified agricultural use within the cultivation of erosion-susceptible monocultures such as maize [2] along the banks of diverted and straightened rivers and streams contributes to this input. This effect is amplified by increased runoff and more severe floodings due to increased heavy rainfall events.

This causes the gravel spawning beds of the brown trout to colmatize. The pores of these well flowed gravel gap systems of pure 2 mm clasts are clogged by the fine sediment particles and thus, the water and gas exchange in the sediment is reduced [3]. In addition, the increasing future water temperatures [4] will intensify this effect by the metabolic activity of algae and other microorganisms. For the lithophilic brown trout, which directly depends on the gravel spawning sites, the increased sediment load is a problem that must be taken seriously. Periodically high runoff additionally stresses the vibration-sensitive eggs. The gills of adult animals can even be clogged by fine sediment particles [3]. The consequences are habitat losses, shifts in fish regions, and, most importantly, a major threat to the species itself [6].

This study seeks to develop a method to collect sediment particles transported in the water

column and to investigate their composition in order to protect affected regions in the future. As established in ecotoxicology, the brown trout is seen as a bioindicator. The effect of different particle qualities on the egg development is exemplary tested on the Konstanzer Ach. These sediment qualities differ in the organic content on the one hand and the proportions of different particle size fractions on the other. This is because the surface area for biological activity by microorganisms increases with smaller grain sizes, while the surface for gas exchange between egg and water decreases. Whether the organic compounds as a nutrient medium for such microorganisms or the small grain size as a surface of attack has a stronger effect on the spawning development, will be investigated in this study. Furthermore, apart from this chemical stress potential, the mechanical one due to particles smaller than the micropores of the egg shell, hence the chorion will be taken into account.

The results can be used to estimate the success of future in-situ-floating incubator campaigns (ISFIC) at certain locations. In the latter the spawn is placed onto the perforated plates of a hatchery box. After the yolk sac has been consumed, the larvae are then released into the water body. These ISFICs consequently are a sustainable alternative to the currently widespread stocking measures with adult fish from aquaculture. The overall aims are firstly quantification and qualification of the future selection criteria regarding erosion for the site of incubation of the spawn as a bioindicator, as it was termed by Pander and Geist [7]. Then, consequently, increasing the ISFIC's success. And thirdly, developing an easy approach to dynamic sediment sampling in general. The results and especially the development of a Citizen-Science suitable sampling method can help to better protect flood-prone regions in the future. According to Speerli et al.'s literature study on the effects of climate change on sediment transport there is an urgent need for research regarding sediment dynamics [8].

2. Material and Methods

2.1 Sediment sampling

2.1.1 Construction of sediment trap

A Sediment Trap (ST) for sample collection was developed. The STs consist of Euroboxes B with 5.1 l volume and a baffle system screwed into the corresponding surplus system lid with alternating flow openings at the top and bottom. The latter have a cross-sectional area of 8.5 cm² similar to the drainage opening. A Blender simulation (Fig. 2) confirmed the effectiveness of this system. The grids guarantee that no larger flotsam such as leaves, but only the entrained sediment, is collected in the ST. The ST should be suitable for Citizen Science in order to apply the sampling method on an

even larger scale. In design, this aspect was prioritized over effectiveness regarding the targeted sample quantity. The ST developed is characterized especially by its easy handling and the low cleaning effort, which also allows larger collaborations for data collection. In addition, the potential sample pool is majorly increased due to the the adaptation to ISFICs and thus increased man-power with the tandem design to the in-situ-floating incubator (ISFI) with the clamp attachment to the floating body. The sediment samples used in this work were collected at short control intervals of 48 hours from the collaboration with the fishing association at the Konstanzer Ach during the during the ISFIC there.



By the positioning in the cross section of the watercourse, the influences of heavy rainfall and flood events on sediment load, its quality and spawning development can be estimated and the sediment load and mortality more reliably compared. At the same time, however, it should be noted as well that the transported sediment is measured only in longitudinal direction and not vertically or laterally. In order to estimate the stocking with ISFIs in terms of future restoration measures, hence improving the stocking success, this in contrast to the research carried out with the HydroEcoSedimentary tool (HEST) [15] is not mandatory, however.

	Sediment Trap (Prillwitz)	HEST (Casas-Mulet et al.) [15]
Sampled spot on a body of water	3	9, more data
Measurement of transported sediment	-in longitudinal direction -on surface and at the bottom	-in vertical, longitudinal and lateral direction -at the bottom
Data acquisition	daily	once-only during dismanteling the experiment
Generated sediment database	more	small
Change	little effort, tested with fishing associations	complex
Application	incubator actions, controlled environment regular ,,sort out"	natural conditions, uncontrolled circumstances, no "sort out"
Purpose of results (for renaturation measures)	assess success of stocking	evaluation of sediment movements

Fig. 3 Literature comparison of ST

In literature comparison with the HEST, the ST developed by Casas-Mulet et al. [15], the simple practical design of the ST offers a great advantage for large-scale data collection. For such, the ST was used and tested in preliminary tests with other fishing associations at the the Friedberger Ach, Prien, Moosbach and Murn.



section of map of water bodies in Bavaria, LfU \bigcirc SP1 \bigcirc SP2

2.1.2 Sampling

The ST was attached to the ISFI with an angular support and a clamp (Fig. 1). During measurements, the ST was recovered and the water-sediment suspension contained was poured into buckets and stored at 5 °C. To calculate the error bars, the STs were each sampled for one hour with a filter fabric of 50 μ m connected to the outflow. At the end of the ISFIC, they were collected from the volunteers, dried and weighed.

2.2 Experimental Set-Up



The selected treatments aim to mimic environmental conditions. Consequently, the fact that the samples are of environmental character, put out on the sampled eggs in water relevant concentrations of 19 mg on average, should be stressed. Nagel et al. also used a similar mimicry in their study of emergence and development of nasal larvae under the influence of various sub-

strate compositions [11]. The spawn should be exposed to clastic sediments of different grain size fractions and organic material in the form of crushed foliage (Fig. 6). Since the small grain size fractions were of particular interest, the grains of the grain sizes 125 - 63 and $< 63 \mu m$ were selected for the treatments. In addition, a treatment with the most frequent grain size fraction from the Konstanzer Ach samples of $250 - 150 \mu m$ was created. Furthermore, the sediments used for the treatments were tested on organic and calcareous shell content. Analogously, sediments from the Friedberger Ach, another water body, were investigated. The latter has a higher abundance of carbonates than the Konstanzer Ach, as the lime deposits at the the incubation box in the field trials suggest.

2.2.1 Treatment selection

Time of application - Especially in early development stages, the spawn is particularly sensitive to external factors [12]. Therefore, the application times were assigned to the first half of development between being green and reaching the eyed ova stage (EOS). An egg is called "green" directly after fertilization, in the EOS the eye pigments are already visible. The fry must not be moved after 24 hours since spawning until reaching the EOS. As a variable to assess the egg development, one is using day degrees which define the product of the temperature in °C and the number of days at this temperature. In order to be able to assess the development of the EOS. Considering the fact that spawning development from the EOS onwards is less dependent on environmental factors [12] the aim here was to assess the development of chorion quality of the longer resting period of the second deployment.

Day Degrees = Temperature in °C x Days

Grain size - As especially smaller grain sizes offer an increased surface for the settlement of microorganisms, particularly the fine fractions were of interest. Also, according to Schmied et al. [11], the chorion does not have micropores > 1 μ m, which is why merely the fines of the clay fractions < 2 μ m would theoretically have the opportunity to penetrate the chorion and thus have an increased damage potential. In order to assess the results with respect to the grain size, the grain size distribution of the sediment samples to be used was examined by sieving and laser granulometry. The samples were dried for 24 hours at 70 °C. The dry crusts formed were removed by mortaring and the samples then sieved by hand through a sieve tower with mesh sizes of 500, 250, 150, 125 and 63 μ m. Subsequently, for each of the required particle

sizes a quarter of the sample was separated. By adding water of hardness 14 in a ratio of 1:1, the sediment samples were previously homogenized by careful stirring, so that the capillary forces prevented the particles from sorting themselves as they do in the dry state. Additionally, these results were veri- and speciefied with the CILAS 1180 laser granulometer of the manufacturer Quantachrome Particle Measurement Technology at the AWI on Sylt. For this purpose, sediment was added until an optical concentration of 100 to 300 was present in the circuit. In cases the sample was too small to reach this concentration, it was processed with its total amount.

Organic and lime scale content - To examine the effects of an increased organic content, the biological activity of microorganisms in particular, the spawn should be exposed to subsequent-ly added organic matter. This is because the saccharides contained in the foliage create a good environment to provide the nutrients for the former. Moreover, as mentioned at the beginning, the sediments of the Friedberger Ach should also be used to determine hints on the potential influence of dissolved carbonates in the water. E.g. the question of whether such an increased ion concentration in the environment would be sufficient to cause an osmotic shock arises. To evaluate the potential influence of the sediment fractions already present in the samples of these fractions, the sediment samples with sufficient material remaining after granulometry, were next muffled for four hours at 550 °C in the Nabertherm B410. After cooling for half an hour in the exicator this was repeated for two hours and 950 °C to dissolve the lime shells as well. The organic content in the grain size distribution was then determined under the assumption of a negligible lime and ash content by a further laser granulometric examination of the samples. An investigation of the organic particle sizes could not be carried out due to the fact that the processing of the laser granulometry results is based on the spherical ideal.

2.2.2 Preparation





Treatmentwise, 38 mg of the respective suspension was individually weighed into 5 ml Eppendorf tubes. Ideally, this corresponds to the 19 mg dry weight of the grain size 125 - 63 μ m, which was used here as a reference, since this the largest proportion of the sample with an average of 28.9 % and also combined with the additional organic treatment. This method was repeated for the organic treatment with 9.5 g each. For this purpose, previously ground and components larger than 500 μ m were sieved off. The errors constisted of 4 % for the fraction < 63 μ m, 5 % for the fraction 63 -125 μ m, 6 % for the fraction 150 - 250 μ m and 10 % for the organics results from the checkweighing of 10 Eppendorf tubes of each treatment, in each case at the beginning and end of of weighing samples.

2.2.3 Egg handling

The eggs are placed on perforated plates in plastic aquariums (Fig. 8, 9) and pipetted once from above with sediments of different grain size fractions, as well as organic material. After hatching, the yolk sac larvae can pass through the holes in the plate into the the lower part of the aquarium. Spatially, the eggs are separated by a perforated grid with a diameter of 10 mm (Carbon filter Airgoclean 100 E) in order to prevent the results from being distorted by the spread of fungi. This procedure has also proved successful in the literature, with the "egg sandwich" used by Pander et al. [9], and its use in Sternecker et al. in the study of factors influencing spawning development in the river substrate [10]. The water of all aquaria was changed and continuously renewed by a combination of overflow and an EHEIM air 400 pump (4 Watt) in a single circuit. Temperature, pH value, oxygen content and electrical conductivity averaged 7.3 °C, 8.1, 10 mg/l and 543 μ S/cm, respectively.



Fig. 8 Set-up aquariums in recirculating system: 1 single treatmentlimited aquarium 2 inflow 4 spillway 5 drain 6, 7, 8 profile plate with perforated plate and eggs 9 gutter 10 pump 11 water reservoir



2.2.4 Deployment

The green spawn had 82 degrees per day to acclimatize before the first treatment was applied, which is approximately 30% of the day degrees to the EOS. The second treatment was applied after 127 day degrees, at approximately 50% of the day degrees to the EOS. The treatments were deployed onto the individual eggs with LDPE pipettes. To the human eye, a precipitate of the pipetted particles was always visible on the egg shell. Nevertheless, there also could be a not inconsiderable precipitation of sediments on the bottom of the aquarium. To quantify the particles effectively adhering to the chorion and the accumulated proportions, the selected dead eggs were dried for 24 hours at 30 °C and then dried for 3 hours at 550 °C. Similar methodology applied for the particles deposited on the perforated plate and the bottom of the aquarium.

2.3 Data analysis

The data was analyzed using the programming language Python.

2.3.1 Sediment composition

The results of the laser granulometric investigations were available as a csv data set. The definitional classification of the investigated sediments was done by the Excel application GRADISTAT [13].

2.3.2 Egg development

Regarding the mortality rates, a distinction was made between fungal, clotted, and burst eggs, as well as larvae that were malformed or died during hatching. The treatments were compared in an ANOVA test after testing the normality distribution and the homogenity of variance.



3. Results

3.1 Sediment composition

3.1.1 Sediment trap as a sampling method

The cooperation with the fishing associations went smoothly. The accuracy of the results, however, depends on the control interval the persons involved adhered to. With longer intervals, such as the five to ten days at Moosbach and Murn algal blooms are likely occur more frequently. In the carbonate-rich Friedberger Ach, there was also precipitation of lime deposits on the baffle plate. The standard deviation determined with the control filter varies - depending on the sediment composition - from water body to water body. The ST furthermore was redesigned for flood events due to the course of the increased flood events during the test phase. The front and rear walls were raised up from the bracket to the flow opening and a slot was added to secure the ST with a tension strap.



Fig. 14 Optimization ST after the Prien flood

3.1.2 Shares of organic compounds and lime shell fragments

The samples from the Konstanzer Ach showed high shares of organic compounds with 63.8 % on average. 28.2 % of the total 27 samples consisted of lime shell fragments (Fig. 10). In the distribution over the sieved particle size fractions lime shell and organic content increase in percentage with decreasing particle size (Fig. 12). The Friedberger Ach samples are dominated by lime shells with 63.4 %, while the organic content is rather low at 10.1 % (Fig. 11). The eggs of the Friedberger Ach treatments were therefore exposed to a higher calcium content in the sediment. Furthermore, the reddish coloration of the Konstanzer Ach samples indicated the content of iron. The samples of the Konstanzer Ach were dominated by particles of classes 98 to 110 [13] with more than 2.5 %, which corresponds to the size range 27 to 62 μ m. It is thus in the investigated range of the water column and silty to sandy-silty. Grains < 1 μ m are present here at about 1.9% (Figs.15, 16, 17). The grain sizes of the Friedberger Ach samples are characterized by the comparatively high proportions of up to four of the classes 99 to 115 and thus of the size range 29 to 88 μ m. The shares of the range < 1 μ m amount to a total of 1.7 % (Figs. 15,16,17). All in all, the KonstanzerAch has a higher proportion of smaller grains than the sandy-silty Friedberger Ach.



Also, the difference of the laser granulometry data before and after muffling the samples, which inversely depicts the organic and calcareous shell fractions was calculated. However the negative ranges were probably caused during the muffle as clay particles transform to coarser lumps as both the consistency as well as the microscopic examination of the samples indicate. In this regard, the differences have to be evaluated cautiously, since the differences in the smaller grain size classes could have arisen due to "caking losses". This results in a negative balance for the Konstanzer Ach and a positive one for the Friedberger Ach. Thus organic matter and calcareous shells seem to influence the grain size distribution in the Friedberger Ach in contrast to the Konstanzer Ach.





Fig. 17: Grain size distribution Friedberger Ach





Fig. 19 Egg development: egg "green", t1



Fig. 20 Eyed ova stage, t2

Fig. 18 Shares of differnt causes of death of the three times of detection

Fig. 21 Yolk sack larvae and empty chorion, t3

3.2 Egg development

3.2.1 Time of death

The highest mortality rates occurred at the beginning of spawning development, which was characterized by fungi as the main cause of death. In the second selection interval in contrast, eggs denatured by water penetration dominated. In the third, they were joined by burst ones before hatching and malformed larvae during hatching. However, the latter were rather rare.

3.2.2 Mortality rates related to treatments

In the analysis of variance, four out of five treatments for the Konstanzer Ach were statistically significant. Here the mortality rates for the grain sizes 63 - 125 and $< 63 \mu m$ were highest at the second time of exposure. This is followed by the treatment 150 - 250 μ m and 63 - 125 μ m at the first exposure. The latter treatment, however, is statistically significant because of its low mortality rate in contrast to the other treatments of the Konstanzer Ach because of the low mortality rate in relation to the control treatment. That of the grain size $< 63 \mu m$ at the first exposure did not prove significant at all, although the mortality rate is 5% higher than that of the control.

Therein, the mortality distributions are not proportional to the masses of particles attached to the chorion per treatment (Fig. 24). For the treatment $\leq 63 \mu m$ at the first time of exposure, for example, the mass is just almost 6.5 % of the original treatment, while the only treatment that did not become significant was the treatment of the Konstanzer Ach $< 63 \mu m$ at the first exposure time the largest mass of particles accumulated in the chorion in this treatment group. In terms of interpretation, however, it should be noted that these results are based on only 10% of the sampled treatment group.



Fig. 22 Overview Mortality per Treatment (FA = Friedberger Ach, KA = Konstanzer Ach, $s3 = grain size 250 - 150 \mu m$, $s5 = 125 - 63 \mu m$, $s6 = < 63 \mu m$, t1 = time of exposition 1, t2 = time of exposition 2, org = additional organic matter) statistically significant compared to control: KAs3t1, KAs5t2, KAs6t2

This discrepancy between mortality rates and weighing results can also be found in the treatment group of the Friedberger Ach. Regarding the mortality rates, however, a trend towards higher failures can be seen for grain sizes < 63 μ m, followed by 150 - 250 μ m and finally 63 - 125 μ m for both exposure times.



Fig. 23 Shares of particles sedimented on the ground of the aquarium (Boden), the perforated plate (Blech), rest (Rest)

Fig. 24 Share of accumulated mass on chorion

This order is also confirmed by the counterbalance with the particles fixed on perforated plate and aquarium bottom fixed particles, with proportions of 16, 9 and -3 % (Fig. 23) of the remaining treatment, whereby the negative balance can be explained by measurement inaccuracies. That the treatments combined with organics show such a high accumulated mass is due to the double amount of treatment.

4. Discussion

4.1 The sediment trap as a sampling method

In order not to endanger the live-character of the results by algal blooms, the control intervals should be kept as short as possible. The standard deviation of the effectiveness of the sediment trap for a sampled water body must always be determined for the later evaluation of the data.

4.2 Sediment composition

Both the treatments < 63 μ m of Friedberger and Konstanzer Ach contained grains < 1 μ m with 1.74 and 1.97 % grains of < 1 μ m contained. Thus, a penetration of the micropores < 1 μ m, as well as corresponding damage due to the mechanical stress would be possible. Since this proportion is larger for the treatments of the Konstanzer Ach, it could also explain the high mortality rates compared to the Friedberger Ach and the corresponding statistical significances. Moreover, smaller grain sizes offer microorganisms such as fungi a greater surface of adherence, so that the oxygen supply is additionally blocked and chemical stress created. The higher mortality rates due to fungi in the earlier development also hint to that fact. In addition, the sediments of the Konstanzer Ach seem to have a stronger influence on the egg development than those of the Friedberger. Apart from mechanical stress as a non-quantifiable side effect, that might be related to the high shares of organics.

4.3 Egg development

Furthermore, the second exposure seems to have a negative influence on the egg development as well, which could be explained by the structural changes of the chorion during the first days after fertilisation and better adherence of particles such as fine sediments. At hatching, the egg shell is separated by the release of an enzyme secretion starting at the head of the larva. Accumulated foreign bodies such as the particles of the treatments could interfere with this process, resulting in bursting.

5. Conclusions

The treatments of the Konstanzer Ach had a more negative influence on the egg development than those of the Friedberger Ach. Thus, apart from the increased potential of mechanic stress within the more prominent share of grain sizes $< 1 \mu m$ there is a chemical one due to the majorly increased share of organic compounds. Because of the not statistically significant differences between the treatments of different grain sizes the latter seems to have a bigger influence

on the egg development in comparison. The results of this study contribute to the evaluation of the impact of different sediment compounds and therefore to the success of future ISFICs. The auxiliary function of these can be used as a sustainable alternative to stocking with adults animals for the restoration of native brown trout populations in combination with renaturation measures. The methodology can also be applied to any other water body. As there have been proposals from Swiss and Austria, offering to collaborate, this study could be extended to the comparison of different water bodies.

Since the data generation with the sediment trap has proven to be very efficient, the field sampling was repeated this year. The "Gewässerkundlicher Dienst", the official agency that oversees this section of the river requested the data to compare it to their own. That is because a sediment monitoring has been underway there since the beginning of this year until 2025. The same length of cooperation for field sampling is intended to continue this work as a long-term trial. This way, the effects of flood events on the sediment composition can be determined and the results compared to those of the hydrographic service. Through this characterization of the sediment load and its quality dynamics major contributions to the conservation of the brown trout and the planned renaturation are made. Fine sediment inputs due to non-compliance with

riparian strips and increased abundance of organic matter due to extensive grassland cultivation are investigated as a source of erosion at various locations and the need of action is identified. In the long term, this helps reducing the consequences of increasing regional flood and drought events, thus counteracting the consequences of man-made climate change.



Fig. 25 Flood during ISFIC at Konstanzer Ach 2022



Fig. 26 Wind erosion due to drought at Friedberger Ach 2023

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