Science of the Total Environment 755 (2021) 142547

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# Can top-down effects of cypriniform fish be used to mitigate eutrophication effects in medium-sized European rivers?

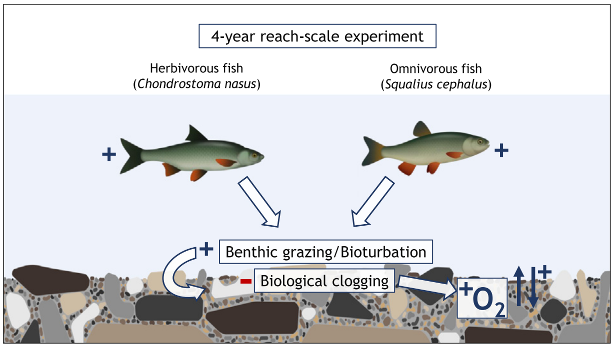
Madlen Gerke [a,1], Dirk Hübner [b], Jörg Schneider [c], Carola Winkelmann [a,*]

[a] University of Koblenz-Landau, Institute for Integrated Natural Sciences, Koblenz, Germany
[b] Bürogemeinschaft für fisch- und gewässerökologische Studien, Marburg, Germany
[c] Bürogemeinschaft für fisch- und gewässerökologische Studien, Frankfurt, Germany

## HIGHLIGHTS

- Eutrophication in running waters causes oxygen depletion in the hyporheic zone.
- Fish stock enhancement increased hyporheic oxygen supply and water exchange.
- Fish stocking did not necessarily decrease periphyton biomass on the river bed.
- Biomanipulation has the potential to mitigate eutrophication effects in rivers.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

*Article history:*
Received 6 May 2020
Received in revised form 14 September 2020
Accepted 18 September 2020
Available online 28 September 2020

Editor: Sergi Sabater

*Keywords:*
Biomanipulation
Benthic grazing
Hyporheic zone
Biological clogging
*Chondrostoma nasus*
*Squalius cephalus*

## ABSTRACT

Eutrophication seriously threatens the ecological quality and biodiversity of running waters. In nutrient-enriched streams and shallow rivers, eutrophication leads to excessive periphyton growth and, in turn, biological clogging, oxygen depletion in the hyporheic zone and finally a reduction in the hyporheic habitat quality. Top-down control of the food-web by manipulating fish stocks, similar to the biomanipulation successfully applied in lakes, offers a promising approach to mitigating the effects of eutrophication in shallow rivers, especially those in which major reductions in nutrient input are not feasible. We conducted a reach-scale experiment over 4 years in a medium-sized eutrophic river to assess whether the top-down effects of two important large European cypriniform fish species, herbivorous common nase (*Chondrostoma nasus*) and omnivorous European chub (*Squalius cephalus*), would mitigate the effects of eutrophication. The enhancement of fish stocks was expected to reduce biological clogging, via the top-down control of periphyton by benthic grazing and enhanced bioturbation, thus increasing oxygen availability in the hyporheic zone as well as water exchange between the surface water and the hyporheic zone. As expected, enhancing the stocks of nase and chub increased both oxygen availability and vertical exchange flux of water in the upper layer of the hyporheic zone. However, periphyton biomass (chlorophyll *a*) was significantly reduced only in deeper pool habitat. Thus, while experimental biomanipulation in a shallow river significantly mitigated the effects of eutrophication in the hyporheic zone, top-down effects on periphyton biomass were rather small. Overall, to our knowledge, our results provide first evidence that the biomanipulation achieved by enhancing herbivorous and omnivorous fish stocks has the potential to mitigate the effects of eutrophication in medium-sized European rivers.

---

[*] Corresponding author at: University of Koblenz-Landau, Institute for Integrated Natural Sciences, Universitaetsstrasse 1, 56070 Koblenz, Germany.
*E-mail address:* cawinkelmann@uni-koblenz.de (C. Winkelmann).
[1] Present address: Bavarian Environment Agency, Wielenbach, Germany.

https://doi.org/10.1016/j.scitotenv.2020.142547
0048-9697/

## 1. Introduction

Eutrophication is a world-wide problem that seriously threatens the ecological quality of surface water bodies by promoting the excessive growth of algae (Smith et al., 1999). Nutrient enrichment has been identified as one of the major stressors on aquatic biota in European streams and rivers (Dahm et al., 2013; Hering et al., 2006). In Europe, 70% of the land area is predicted to contain river catchments with a high potential to exhibit undesirable periphyton growth due to nutrient enrichment (McDowell et al., 2020). In such nutrient-enriched streams and shallow rivers, intense periphyton growth negatively affects physical-chemical surface water quality by causing large diel fluctuations in oxygen concentrations and pH (Smith et al., 1999). Moreover, eutrophication-driven periphyton blooms cause biological clogging of the hyporheic zone (Hartwig and Borchardt, 2015; Ibisch et al., 2009). External clogging by benthic algal mats on the riverbed reduces the water exchange between surface water and the hyporheic zone (Ibisch et al., 2009). Following the detachment of algal mats, internal clogging by infiltrated dead algal cells both reduces streambed permeability and increases biological oxygen demand in the hyporheic zone, by enhancing the decomposition of decaying algae (Hartwig and Borchardt, 2015; Ibisch and Borchardt, 2002). The result is oxygen depletion in the hyporheic zone, which in turn strongly reduces the habitat quality for gravel-spawning fish and sensitive invertebrates, such as juvenile freshwater mussels (Geist and Auerswald, 2007; Keckeis et al., 1996).

There are two principal approaches to controlling eutrophication: a reduction of its sources and a mitigation of its effects. The reduction of phosphorus input, being the limiting nutrient in most European rivers (McDowell et al., 2020), is generally regarded as a key tool for controlling eutrophication. In the past four decades, there have been successes in reducing phosphorus inputs from point sources (e.g. through enhanced wastewater treatment) and diffuse sources (e.g. through riparian buffer strips) (Jarvie et al., 2013). However, in many cases no reduction in nuisance algal growth occurred after reductions in river water phosphorus concentrations (Bowes et al., 2012; Jarvie et al., 2013). One difficulty in this context is that the reduction of nutrient input is less likely to substantially decrease periphyton biomass accrual unless nutrient concentrations in the river are reduced below algal growth-limiting threshold concentrations (e.g. threshold of total phosphorus in temperate streams and rivers derived from regression analysis: 43 $\mu g\ L^{-1}$; Dodds et al., 2006, 2002). Reducing nutrient concentrations to limiting levels is expensive and difficult to achieve, especially in catchment areas which are densely populated and include a high level of agricultural activity (Bowes et al., 2012). In streams, light limitation by riparian canopy may be an effective tool to control eutrophication (Burrell et al., 2014; Ghermandi et al., 2009). However, a full canopy cover can rarely be reached in wider rivers and periphyton growth is therefore not exceedingly light-limited during the vegetation season. Another possibility is to mitigate the effects of eutrophication through top-down control of the food web by manipulating fish stocks. Specifically for standing waters, the most common biomanipulation method is the active removal of zooplanktivorous fish or the stocking of piscivorous fish in order to reduce predation pressure on zooplankton communities and thereby enhance their grazing on phytoplankton (Benndorf, 1990; Hansson et al., 1998). However, the same approach cannot be applied to running waters, because their food web structure differs from that of standing waters. Unlike in standing waters, the main consumers of autotrophic biomass in streams and shallow rivers are benthic grazers such as macroinvertebrates and herbivorous fish (e.g. Power, 1990; Power et al., 1985). An increase in benthic grazing therefore requires either an increase in the stock of herbivorous fish or a reduction of predation pressure on benthic invertebrate grazers.

Benthic grazing generally exerts strong top-down effects on periphyton in running waters (Feminella and Hawkins, 1995; Hillebrand, 2009). Herbivorous fish in North American streams were found to reduce periphyton biomass (e.g. Gelwick and Matthews, 1992; Stewart, 1987). There is also evidence for the indirect top-down control of periphyton in running waters via three- or even four-level trophic cascades, with the latter consisting of omnivorous predatory fish, predatory insects, invertebrate grazers and periphyton (three-level cascades: e.g. Winkelmann et al., 2014; four-level cascades: e.g. Power, 1990). The enhancement of fish grazing by stocking herbivorous fish may therefore mitigate the effects of eutrophication in shallow rivers, with additional indirect effects induced by an increase in invertebrate grazing through trophic cascades (Gerke et al., 2018). In addition to trophic interactions, the bioturbation resulting from the activity of benthic foraging fish could increase the water exchange between surface water and the hyporheic zone due to a reduction of clogging and thus contribute to mitigating eutrophication effects in rivers. Benthic-foraging cypriniform fish were shown to increase the mobility of sediments, resulting in substrate coarsening in the upper layer of the hyporheic zone (e.g. Pledger et al., 2017, 2016).

European rivers are home to two fish species with a high potential for mitigating the effects of eutrophication: the large herbivorous cypriniform common nase (*Chondrostoma nasus*) and the large omnivorous cypriniform European chub (*Squalius cephalus*) (Leuciscidae: Leuciscinae). Both species are typical members of the fish communities in the hyporhithral and epipotamal zones of European rivers, specifically in Central and Eastern Europe where the distribution ranges of both species overlap. In Central Europe, the rheophilic common nase was once one of the most abundant and productive fish species in these river zones (Peňáz, 1996). Despite large-scale population declines, the nase remains one of the most abundant fish species in many European rivers (Reckendorfer et al., 2001). It is the only fish species in European rivers that is specialised for feeding on periphyton, preferentially on benthic diatoms (Corse et al., 2010; Vater, 1997). Nase scrape periphyton from coarse substrates, leaving characteristic grazing scars (Freyhof, 1995). Adult fish typically form large single-species shoals ranging in size from a dozen up to several hundred individuals that move actively within defined home ranges (Huber and Kirchhofer, 1998; Lusk, 1967). In contrast to the highly specialised nase, European chub is a generalist, able to feed on periphyton, especially filamentous algae, and on benthic invertebrates (Balestrieri et al., 2006; Hellawell, 1971). Chub of larger size classes prey on small fish such as common minnows (*Phoxinus phoxinus*) and bullheads (*Cottus gobio*) (Mann, 1976; Hellawell, 1971).

In a previous short-term field mesocosm experiment using 8-$m^2$ enclosures, the top-down effects of both fish reduced hyporheic oxygen depletion, suggesting a reduction of biological clogging attributable to benthic grazing (nase) and bioturbation (chub) (Hübner et al., 2020). This result suggests that the simultaneous enhancement of nase and chub stocks can induce synergistic top-down effects (Hübner et al., 2020). However, the top-down effects observed in mesocosm experiments, where foraging by fish is concentrated in a small area, might be less relevant at larger, realistic scales (Gil et al., 2016). In the present study, we conducted a 4-year reach-scale experiment in the hyporhithral zone of a medium-sized eutrophic gravel-bed river to assess whether enhancing nase and chub stocks offers an effective ecosystem-scale strategy for mitigating the effects of eutrophication in the hyporheic zone. Based on the results of the mesocosm experiment, we expected that the enhancement of fish stocks would reduce biological clogging of the hyporheic zone via the top-down control of periphyton and by enhancing bioturbation. Specifically, we hypothesised that the enhancement of fish stocks would increase both the dissolved oxygen concentration and the vertical exchange flux of water in the hyporheic zone in addition to reducing periphyton biomass on the riverbed.

## 2. Material & methods

### 2.1. Experimental site

The reach-scale experiment was conducted along a 1.4-km long stretch in the hyporhithral zone of the Nister River (Rhineland-Palatinate, Germany, 50°43′24 N, 7°44′24 E), a medium-sized gravel-bed river with a catchment area of 246 km$^2$ (catchment area supplied by the State Office of Survey and Geobasis Information of Rhineland-Palatinate). Following the broad typology of European rivers developed by Lyche Solheim et al. (2019), the Nister River is assigned to type R-08 (Mid altitude, siliceous, medium-large rivers). Mid-altitude siliceous or calcareous rivers in this size range are generally common in Europe (river types R-08 and R-10: 6652 water bodies; 8.6% of all river water bodies assigned to a broad river type according to the typology of Lyche Solheim et al., 2019).

The river's hydrological regime is strongly rainfall-dependent and generally follows a seasonal pattern, with maximum discharge in winter and minimum discharge in summer (average mean discharge winter: 6.3 m$^3$ s$^{-1}$, summer: 2.4 m$^3$ s$^{-1}$; measured approximately 4 km upstream of the experimental site, at gauge Heimborn, ID 2724030100; data supplied by the State Office for Environment of Rhineland-Palatinate). Land-use in the catchment area is dominated by forestry (48%), pasture (26%) and agriculture (16%) (Local Environmental Agency, SGD SGD Nord, 2020). Due to phosphate emissions in the catchment area from several small municipal wastewater treatment plants and diffuse emissions from the different types of land-use, the Nister is highly eutrophic, with the effects including oxygen oversaturation and an extremely high daytime pH, especially during the spring algal bloom (e.g. maximum oxygen saturation in April 2016: 182%, pH: 10.2; Gerke et al., 2018). At the experimental site, forest covered the right bank and pasture the left bank of the river (see pictures in Appendix A). Under flow conditions of approximately 1 m$^3$ s$^{-1}$, the width of the experimental reach averages 14 m and the water depth 0.28 m. The riverbed at the experimental site mainly consists of cobbles (6.3–20 cm) and boulders (20–63 cm).

The benthic algal and cyanobacterial community in the Nister is largely composed of diatoms, but during the summer filamentous cyanobacteria and filamentous green algae (mostly *Cladophora* sp.) may become dominant (Gerke et al., 2018). The community of benthic invertebrates is largely composed of chironomid larvae and scraping grazers (e.g. the mayfly *Ephemerella ignita* and the snail *Ancylus fluviatilis*) (Gerke et al., 2018). The fish species used in the experiment, common nase and European chub, are the most common large fish species in the river, with nase occurring at high natural densities in some sections. According to reports of locals, the natural densities of large fish in the Nister may have been considerably higher around 25 years ago. The densities of large fish in the river may have decreased over the past two decades due to predation by cormorants (*Phalacrocorax carbo sinensis*), which have been observed to forage in the catchment area since the late 1990s. Among the small zoobenthivorous fish, bullhead (*Cottus gobio*), common minnow (*Phoxinus phoxinus*) and stone loach (*Barbatula barbatula*) occur in high densities (Gerke et al., 2018).

### *2.2. Experimental design and fish stock manipulation*

Our reach-scale experiment was conducted in two stretches of the experimental reach of the Nister River in two 2-year experimental phases, following a modified BACI design (Before-After-Control-Impact, Stewart-Oaten et al., 1986). Stocks of nase and chub differed between the two stretches in the first experimental phase (dissimilarity phase, June 2015 – August 2017) but were similar in the second phase (similarity phase, August 2017 – July 2019). The lower stretch (length 550 m) served as the reference stretch, with high fish stocks throughout the experiment, and the upper stretch as the impact stretch (length 595 m), with very low fish stocks during the dissimilarity phase and high fish stocks during the similarity phase (Fig. 1).

This two-phase design allowed us to detect the effects of an increased fish stock regardless of the temporal variability or natural differences in other factors between the experimental stretches. To minimise possible edge effects in the upper part of the reference stretch, the two stretches were separated by a buffer stretch (length 220 m). The design of the experiment differed somewhat from a classical BACI design. First, there was no reference phase comprising natural, undisturbed conditions. Instead, fish stocks were manipulated in both phases to ensure the desired fish densities. Second, the experiment started with the dissimilarity phase and was followed by the similarity phase because the initial natural biomasses per area of nase and chub (> 15 cm) were already higher in the reference stretch than in the impact stretch (reference: 88.5 g m$^{-2}$; impact: 18.3 g m$^{-2}$). Consequently, a large adjustment of the fish stock in the impact stretch was necessary only during the similarity phase.

The upper and lower ends of the reference, buffer and impact stretches were closed off by dynamic fish weirs constructed according to Mühlbauer et al. (2003) to facilitate manipulation of the fish stock. Prior to the experiment, the dynamic fish weirs were installed over the entire cross-profile of the river (see Fig. A.3 in Appendix A). Each weir consisted of a set of linked panels made of PVC pipes (19 mm in diameter) connected to each other at 20-mm intervals, which effectively prevented the passage of large fish (body size >20 cm). The weirs were installed perpendicularly, as shown in Fig. 2, with the lower rim placed more upstream than the floating upper rim. The lower rim of each panel was fixed at the river bottom, while the upper rim was kept afloat on the water surface by floating devices. During low- and mid-flow conditions, the fish weirs were cleaned at 3-day to 2-week intervals, depending on the season, by moving leaf litter and debris from the upstream side to the downstream side of the barrier. The dynamic fish weir is highly resistant to floods because during high water levels and clogging both water and debris are able to pass over the submerged weir without damaging it (Mühlbauer et al., 2003). This implies that the dynamic fish weir does not function as a fish barrier during high-flow conditions (see Fig. A.4 in Appendix A). It was therefore necessary to regularly control and adjust the fish stocks during the experiment, especially in spring following the long winter flood periods.

Stocks of nase and chub (> 15 cm) were regulated at the beginning of each experimental phase and additionally controlled and adjusted three times per year (spring, summer, autumn) by electrofishing (EFGI 650, Bretschneider Spezialelektronik, Chemnitz, Germany). Electrofishing was approved by the fisheries department of the local environmental agency (SGD Nord, Rhineland-Palatinate, Germany). The fish stocks were typically assessed by continuous sampling from the downstream to the upstream weir over the entire length of each experimental stretch in two passes using the mark-recapture method. Using

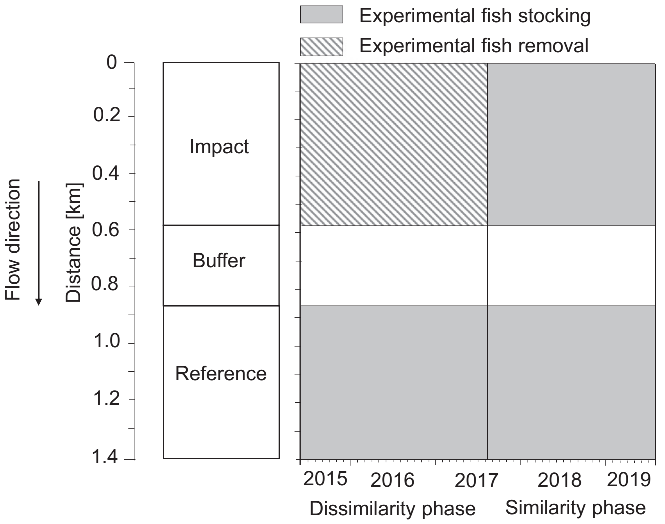


**Fig. 1.** Timetable and spatial set-up of the reach-scale experiment. During the dissimilarity phase, nase and chub were stocked in the reference stretch and removed from the impact stretch. During the subsequent similarity phase, nase and chub were stocked in both experimental stretches in order to achieve similar fish stocks in the reference and impact stretches.

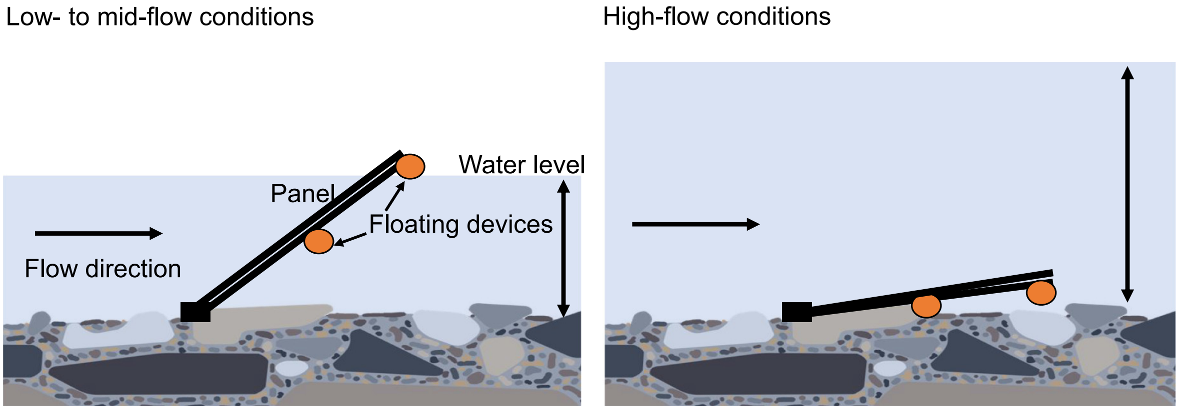


**Fig. 2.** Schematic side view of a dynamic fish weir under different flow conditions.

this method allowed the best possible stock estimation of the mobile shoaling species nase. Each fish (> 15 cm) caught in the first pass was colour-marked using a needleless injector (Dermojet, Akra DermoJet, Pau, France). The total length of each caught fish was measured to the nearest centimetre. Mortality due to electrofishing was negligible (< 0.1%).

During the dissimilarity phase (June 2015 – August 2017), fish caught in the second pass were removed from the impact stretch and transferred to the reference stretch. After the stocks of nase and chub in the reference stretch reached at least a total of 1700 individuals for both species combined, additional fish were transferred to the buffer stretch. In the impact stretch, depending on the recapture rate in the second pass, a third or fourth electrofishing pass was conducted during the dissimilarity phase to remove as much fish as possible with reasonable effort (maximum one working day). From July 2016 until the end of the dissimilarity phase, in August 2017, removal rather than mark-recapture was used in the impact stretch to ensure maximally efficient fish removal.

During the similarity phase (August 2017 – July 2019), stocks of nase and chub were assessed in both experimental stretches using the mark-recapture method and then boosted in the impact stretch by stocking fish that had been caught directly downstream of the reference stretch. Because the natural fish density downstream of the reference stretch was relatively low, it was necessary to additionally transfer fish from the reference to the impact stretch in order to ensure similar fish stocks in the two stretches. A single electrofishing pass was additionally conducted in the buffer stretch during each electrofishing session in order to obtain a raw estimate of fish densities.

Due to a high abundance of cormorants in the catchment area, the impact of their predation had to be controlled in order to sustain the manipulated fish stocks. Therefore, cormorant management measures (deterrence and lethal shooting) were applied throughout the experiment. Deterrence measures were conducted during daily patrols by volunteers of the local river protection association. Lethal shooting was applied as an aid to deterrence only between August 15th and February 15th outside of the cormorant breeding season in accordance with state regulations of Rhineland-Palatinate.

### *2.3. Field sampling*

In both experimental stretches, field samples were taken in a subsection along a riffle-pool-riffle sequence (length: reference stretch: 90 m, impact stretch: 100 m). Equipment for sampling the hyporheic zone was installed at three different sampling sites within the subsections (A, B and C; Fig. 3). To represent different hyporheic flow conditions, the sampling sites consisted of: (A) a riffle crest, (B) a riffle tail (potential upwelling zone) and (C) a riffle head (potential downwelling zone).

In April 2015, nine multi-level probes (manufactured by the central workshop of the University Kassel, Germany) following Lenk et al. (1999) were installed in the riverbed. They allowed the collection of hyporheic water samples from four different depths (8 cm, 13 cm, 23 cm and 33 cm within the riverbed). The probes were evenly distributed across the three sampling sites in each stretch (Fig. 3). In August 2015, five temperature lances (Hartmann GmbH, Neuwied, Germany) were installed in the riverbed (one at site A, two each at sites B and C) to record the vertical temperature profiles that were used to calculate the vertical flux of water in the hyporheic zone, as a measure of hydrologic exchange. Four temperature loggers (Tidbit v2, Onset Computer Corp., Bourne, Massachusetts, USA) attached to each lance recorded the temperature at depths of 5, 10, 20 and 30 cm within the riverbed at 15-min intervals. The temperature lances were retrieved at the end of each sampling season in October to read the data from the loggers and immediately thereafter reinstalled in the riverbed. Periphyton samples were taken from two sites in each stretch (Fig. 3), one in a moderate- to fast-flowing shallow area (riffle site) and the other in a slow-flowing, relatively deep area (pool site) (see Appendix B for details on water depth and current velocity at the sampling sites).

During the dissimilarity phase, hyporheic water and periphyton sampling started two weeks after the initial fish stock manipulation, in June 2015. Because the initial fish densities in the reference stretch were already considerably high, a longer adaptation period between fish stock manipulation and the first sampling was not necessary. During the similarity phase, sampling started after a 2-month adaptation period following fish stock manipulation. Consequently, samples were collected from October 2017 until the end of the experiment, in July 2019. Hyporheic water and periphyton samples were always collected on the same date, with an interval of one month during the dissimilarity phase and two weeks during the similarity phase. All samples were collected at low- to mid-flow conditions between spring and autumn.

On each sampling date, the multi-level probes were sampled simultaneously in the two experimental stretches by two teams. Water samples (35 mL) from each depth horizon were obtained using a polypropylene syringe to apply a slight negative pressure. The obtained samples were carefully transferred to a 100 mL plastic vessel without shaking to minimise the entry of atmospheric oxygen. Immediately thereafter the dissolved oxygen concentration (DO) and electrical conductivity of each sample were measured (WTW, Multi 3430 with FDO 925 and TetraCon 925 probe, Wissenschaftlich Technische Werkstätten, Weilheim; Germany). In addition to hyporheic water sampling, the physical and chemical characteristics of the surface water (temperature, pH, DO, electrical conductivity) were measured directly in the river at each probe site. At the end of the sampling season in 2016, many of the multi-level probes were clogged at several depths, necessitating their retrieval in autumn 2016 to exchange their clogged porous filters. Moreover, we found that in the impact stretch, the water depth at some

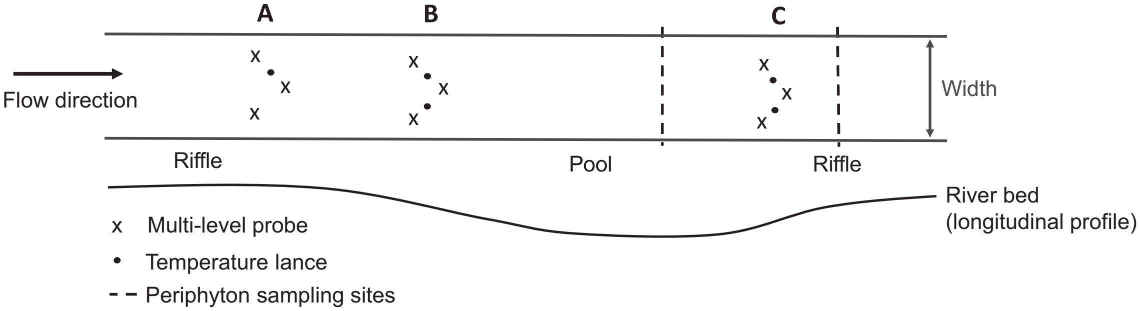


**Fig. 3.** Arrangement of the sampling sites (A, B and C) and measurement equipment in the experimental stretches.

of the sampling sites, originally selected during mid-flow conditions, was insufficient for large fish during the low-flow conditions in summer. This necessitated shifting the sampling sites within the impact stretch to deeper sites. The sampling sites within the reference stretch were shifted as well to ensure equal treatment in both experimental stretches.

For periphyton sampling, ten flat stones of similar texture were randomly chosen over the entire width of the river at each sampling site and pooled to obtain one sample per sampling site. Periphyton was removed from the stones by carefully brushing their surfaces with a coarse brush and collecting the periphyton with river water. The resulting suspensions were kept in the dark by placing them in a cooling box until further processing in the laboratory later the same day.

The hyporheic zone at the experimental site was further characterised by analysing the nitrate concentration in water samples, estimating the fine sediment accumulation rates using sediment traps and assessing sediment composition using the freeze-core sampling technique of Humpesch and Niederreiter (1993) (see Appendix C for details).

### 2.4. Laboratory analyses

Periphyton was characterised with respect to its total biomass (measured as ash-free dry mass, in mg AFDM $cm^{-2}$) and its autotrophic biomass (measured as the chlorophyll *a* concentration in μg Chl *a* $cm^{-2}$). Both were quantified based on the total volume of the obtained periphyton suspension and the sampled area of the stone. The surface area of the stones was estimated by carefully wrapping each stone in aluminium foil, cutting off overlapping areas, and weighing the foil. After their total volumes had been determined, the periphyton suspensions were homogenised using a magnetic stirrer. AFDM was quantified by drying duplicate 10-mL aliquots at 105 °C for 24 h. The dried samples were weighed, ashed in a muffle furnace at 510 °C for 5 h and then reweighed using a microbalance (XS205 Dual Range, Mettler-Toledo, Columbus, Ohio, USA). The Chl *a* concentration was determined in triplicate 2-mL aliquots centrifuged for 3 min at 13,000 rpm (16,060 × g, Micro 200R; Hettich Zentrifugen, Tuttlingen, Germany). The supernatants were discarded, and the pellets stored at −80 °C. Chl *a* was extracted by resuspending the pellets in 96% ethanol containing 1 g $MgCO_3$ $L^{-1}$ buffer and then incubating the samples at room temperature in the dark for at least 3 h. They were then analysed spectrophotometrically (Specord 205; Analytic Jena, Jena, Germany) according to Mewes et al. (2017).

### 2.5. Data analysis

The sizes of nase and chub stocks were estimated using Chapman's modified Petersen estimator (Ricker, 1975). The biomass of individual fish was calculated using the length-weight regressions obtained from our data, based on the weights and lengths (measured to the nearest cm) of nase and chub individuals caught on three different electrofishing dates (autumn 2016, summer 2017, spring 2018). The data were used to obtain a power function relating the total length (L) and wet weight (W) for nase ($W = 0.0073{*}L^{3.0945}$, $r^2 = 0.96$, $n = 799$) and chub ($W = 0.0098{*}L^{3.041}$, $r^2 = 0.97$, $n = 297$). For each electrofishing date and each stretch, the total biomass of the caught fish was calculated by summing the individual biomasses of all fish as estimated using the power functions. To estimate the stock biomass, the mean individual biomass of nase and chub was calculated by dividing the total biomass of the caught fish of one or the other species by the number of the caught fish of that species. The total biomass of a fish stock was calculated by multiplying the mean individual biomass of nase and chub by the respective estimated stock size.

Vertical flux of water was calculated from the temperature time series data according to the amplitude attenuation method of Keery et al. (2007). This method assumes that the vertical flux of water in the hyporheic zone is predominantly vertical, that the physical and thermal properties of the sediment and fluid remain constant over time, and neglects thermal dispersivity. The Matlab program VFLUX 2 (Gordon et al., 2012; Irvine et al., 2015) was used to automatically calculate the vertical flux of water between sensors located at different depths. The values of the physical properties used in the calculations were those suggested in the documentation included with the program: dispersivity, 0.001 m; saturated-bed thermal conductivity, 0.0045 cal $s^{-1}$cm°C; volumetric heat capacity of the sediment, 0.5 cal $cm^{-3}$ °C and volumetric heat capacity of the water, 1.0 cal $cm^{-3}$ °C. Sediment porosity was estimated from the weight loss off the dried freeze-core samples (mean porosity = 0.19, $n = 61$), assuming a grain density of 2650 kg $m^{-3}$ as suggested by Keery et al. (2007). Data subsets whose diurnal signals were below the sensor precision of 0.2 °C as well as data for which the VFLUX2 program calculated a thermal diffusivity outside a plausible range for streambed materials (Irvine et al., 2015) were excluded from further analyses. Amplitudes below the sensor precision usually occurred during winter (December – March) and at the 30-cm depth horizon.

To minimise problems associated with multiple testing, only the response variables most likely to be affected by fish stock enhancement were selected to test our hypothesis: DO (mg $L^{-1}$) at different depth horizons of the hyporheic zone, absolute values of the vertical flux of water between 5- and 10-cm depth (m $day^{-1}$), Chl *a* concentration (μg $cm^{-2}$) and AFDM (mg $cm^{-2}$). The other environmental variables assessed in the monitoring program were only used to additionally characterise the hyporheic zone at the experimental site (see Appendix C for details). Hyporheic DO data were averaged over all probes and sampling sites because the relatively large number of 9 probes allowed us to calculate a robust mean over different hyporheic flow conditions. The difference of the mean values (averaged over all probes) between the impact and the reference stretch on each sampling date was calculated to account for seasonal changes and autocorrelation of the variables. For periphyton biomass, we did not average the data over the sampling sites because we obtained pooled samples from only two different sites. The differences between the impact and the reference stretch on each sampling date were therefore calculated separately for the riffle and the pool sampling sites. As the vertical flux of water was calculated from continuous time series data, the differences between the experimental stretches were calculated based on weekly mean values (averaged over all temperature lances). To test the hypothesis, the mean difference in each variable between the experimental stretches during the dissimilarity phase and the similarity phase was compared. Analysing the differences between the stretches minimised any temporal variability as well as the effects of factors similarly influencing the two stretches (Smith et al., 1993). The effects of the experimental manipulation were registered as a change in the mean difference between the stretches in the similarity phase vs. the dissimilarity phase, tested by randomised intervention analysis (RIA; 10,000 iterations; Carpenter et al., 1989).

The data of hyporheic DO from 2015 showed a clear effect of probe installation (Fig. D.1 in Appendix D). Therefore, only data of hyporheic DO measured starting from spring 2016 were included in the statistical analyses, as they represented undisturbed sediment conditions. This reduced the sample size for hyporheic DO in the dissimilarity phase from 18 to 12. In addition, only vertical flux data obtained starting in spring 2017 could be used in the analysis, because shifting the sampling sites in the two stretches at the end of the season in 2016 apparently resulted in distinct changes in the vertical flux of water (Fig. D.2 in Appendix D). This reduced the sample size for vertical flux data in the dissimilarity phase from 47 to 20. By contrast, there was no distinct change in the hyporheic DO following the shifting of the sampling sites (Fig. D.2 in Appendix D) and the 2016 data could therefore be included in the statistical analyses. At one of the three sampling sites in the impact stretch, extremely high nitrate concentrations and electric conductivity values were measured on each sampling date in 2016 (i.e. before the sampling sites were shifted), suggesting a lateral influx of nitrate-rich groundwater (Fig. D.3 in Appendix D). Therefore, data of hyporheic DO obtained at this sampling site (3 of 9 probes) were also omitted from the statistical analyses.

Statistical analyses and graph plotting were performed using R version 3.6.2 (R Development Core Team, 2019).

## 3. Results

### 3.1. Fish stock manipulation

Overall, fish stock manipulation was successful throughout the experiment. During the dissimilarity phase, mean fish density was ~17-fold higher and mean fish biomass per area ~51-fold higher in the reference stretch than in the impact stretch (Fig. 4, Table E.1 in Appendix E). Although the fish stock in the impact stretch was assessed by the mark-recapture method only on three dates, the numbers of captured fish determined on the other three dates were also very low (mean ± SD: 0.011 ± 0.003 individuals [Ind] $m^{-2}$; see Table E.2 in Appendix E for details). Taken together, these results demonstrated that the fish stock in the impact stretch was very low throughout the dissimilarity phase. During the similarity phase, the differences in fish biomass per area and fish density between the two experimental stretches were much smaller. However, fish biomass per area and fish density were still somewhat higher in the reference stretch than in the impact stretch. During the similarity phase, fish biomass per area and fish density in both stretches were generally lower than in the reference stretch during the dissimilarity phase, due to the difficulties in obtaining enough fish from the natural fish stock of the river. However, fish biomass per area and fish density were considerably enhanced in the impact stretch throughout the similarity phase (20-fold increase in mean biomass per area and 8-fold increase in mean density compared to the dissimilarity phase; Fig. 4, Table E.1 in Appendix E).

### 3.2. Dissolved oxygen concentration and vertical flux of water in the hyporheic zone

Our hypothesis that fish stock enhancement increases hyporheic oxygen concentrations was supported by the experimental results. The mean between-stretch difference in DO at the uppermost depth horizon (8 cm) was significantly higher following fish stock enhancement in the impact stretch during the similarity phase ($p = 0.01$, RIA, dissimilarity phase: $n = 12$, similarity phase: $n = 19$; Fig. 5), indicating a relative increase in the DO in the presence of nase and chub. The increase in the mean between-stretch difference was even more pronounced when the analysis included only the hyporheic DO measured at probe sites located in the middle of the river ($p < 0.01$, RIA, dissimilarity phase: $n = 12$, similarity phase: $n = 19$; Fig. 5).

In deeper horizons of the hyporheic zone (13, 23 and 33 cm), the mean difference in the DO between the two stretches was similar during the two experimental phases ($p > 0.05$, RIA, dissimilarity phase: $n = 12$, similarity phase: $n = 19$; Fig. 5), suggesting that the effect of fish stock manipulation was limited to the upper layer of the hyporheic zone.

Despite the positive effect of fish stocking, the mean hyporheic DO was generally lower during the similarity phase than during the dissimilarity phase, especially in the case of the exceptionally dry summer of 2018, when discharge was consistently low (Fig. 6, Table 1). However, the decrease in the mean hyporheic DO during the similarity phase was smaller in the impact stretch than in the reference stretch (12%

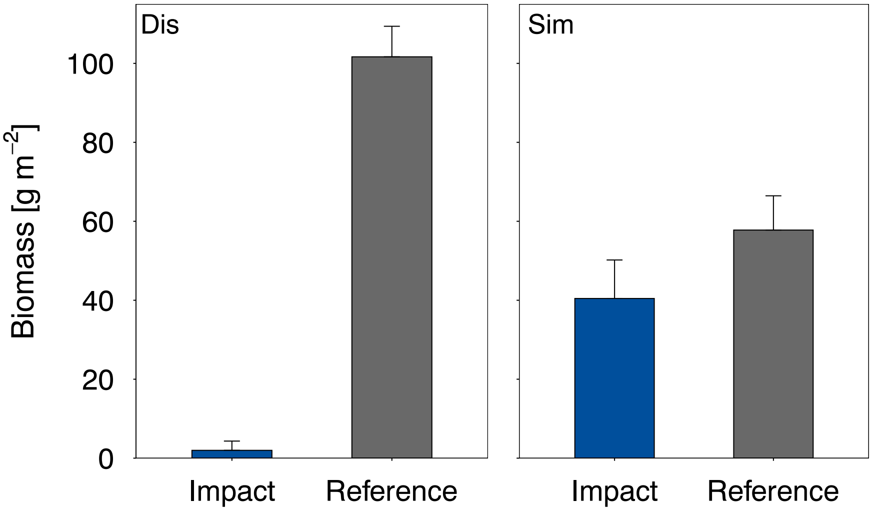


**Fig. 4.** Mean total biomass per area of nase and chub (> 15 cm) in the impact and reference stretches. Left: dissimilarity phase (Dis), impact stretch: $n = 3$, reference stretch: $n = 6$. Right: similarity phase (Sim), $n = 5$. Error bars represent the standard deviations.

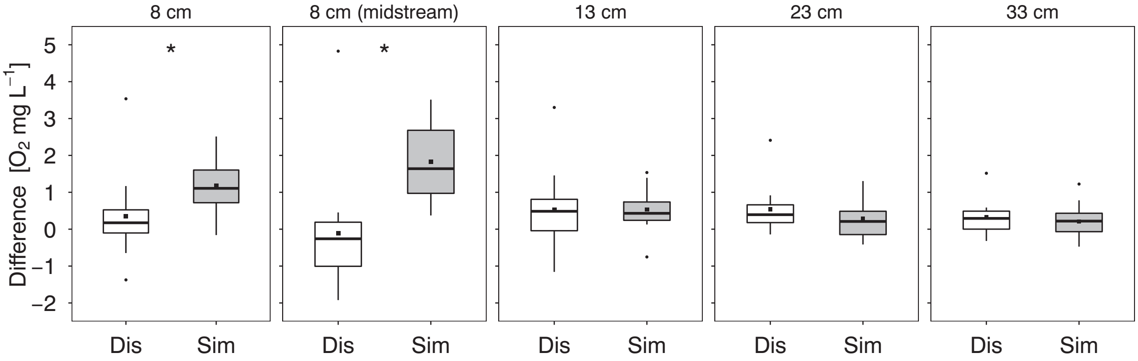


**Fig. 5.** Differences in the oxygen concentrations in the hyporheic zone (8-, 13-, 23- and 33-cm depth; midstream: including only sampling sites in the middle of the river) between the impact stretch and the reference stretch during the dissimilarity phase (Dis; 12 sampling dates) and the similarity phase (Sim; 19 sampling dates). Boxes: 75% and 25%, whiskers: 95% and 5%, squares: mean differences, dots: outliers. *Significant ($p < 0.05$).

vs. 37% reduction at 8 cm depth compared to the dissimilarity phase, Table 1).

During the 4-year experiment, the mean hyporheic DO was generally higher in spring than in summer, indicating a large effect of biological clogging following the spring breakdown of algal biomass (Fig. 6). Fish stocking seemed to have the strongest positive effect on hyporheic oxygen availability during spring, as indicated by the largest between-stretch difference in the DO between May and mid-June, i.e. during the similarity phase (mean difference vs. reference stretch: 2.6 mg $L^{-1}$; Fig. 6, Table 1).

In accordance with the positive effect of fish stocking on hyporheic oxygen availability, the mean between-stretch difference in the vertical flux of water between 5 and 10 cm depth increased significantly following fish stocking in the impact stretch during the similarity phase ($p < 0.001$, RIA, dissimilarity phase: $n = 20$, similarity phase: $n = 37$; Fig. 7), suggesting a relative improvement in riverbed permeability.

### 3.3. Periphyton biomass

Our hypothesis that fish stock enhancement would reduce periphyton biomass on the riverbed was only partly supported. Periphyton biomass in the impact stretch was already lower during the dissimilarity phase (Table 1), resulting in a negative difference compared to the reference stretch. The mean between-stretch difference in autotrophic periphyton biomass (Chl $a$) at the pool site became significantly more negative during the similarity phase ($p < 0.01$, RIA, dissimilarity phase: $n = 17$, similarity phase: $n = 20$; Fig. 8), indicating a reduction of periphyton biomass due to enhanced benthic grazing following fish stock enhancement. The between-stretch difference in total periphyton biomass (estimated as AFDM) at the pool site showed a similar tendency, although the magnitude of the change between the dissimilarity phase and the similarity phase was not significant ($p = 0.06$, RIA, dissimilarity phase: $n = 17$, similarity phase: $n = 20$; Fig. 8). At the riffle site, the between-stretch differences in Chl $a$ and AFDM did not change significantly from the dissimilarity phase to the similarity phase (Chl $a$: $p = 0.6$, AFDM: $p = 0.1$, RIA, dissimilarity phase: $n \geq 17$, similarity phase: $n = 20$, Fig. 8).

## 4. Discussion

In standing waters, the enhancement of grazing pressure by biomanipulation is a well-established technique to mitigate the effects

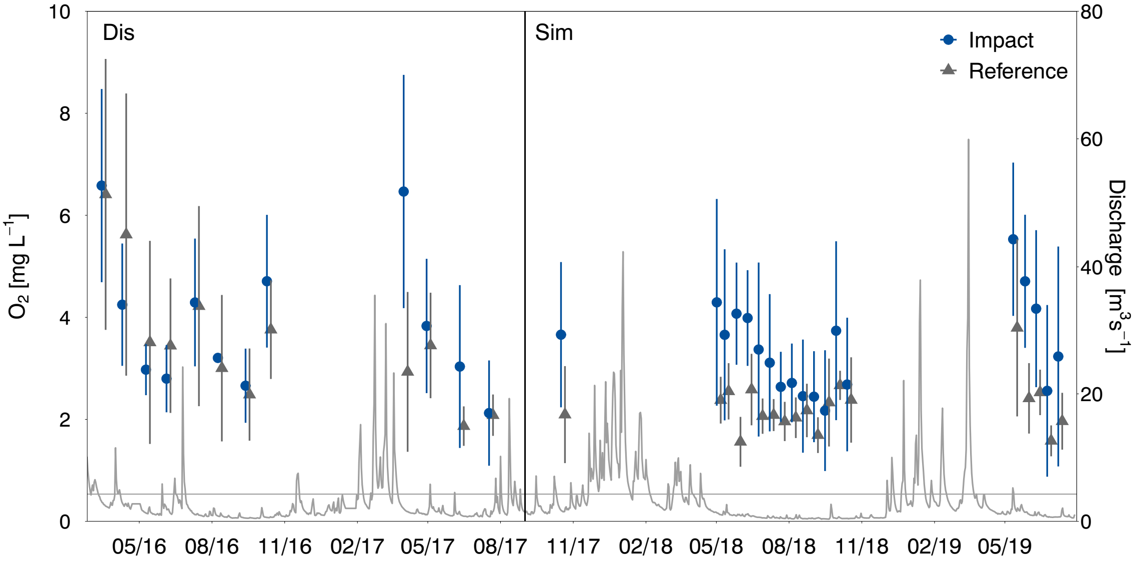


**Fig. 6.** Mean oxygen concentration (left y-axis) in the upper hyporheic zone (8-cm depth) of the experimental stretches during the dissimilarity phase (Dis) and the similarity phase (Sim). The transition between the phases is indicated by the black vertical line between 07/17 and 10/17. Error bars represent the standard deviations; $n \geq 4$ samples except for one sampling date in August 2016 (impact stretch: $n = 2$, reference stretch: $n = 3$). The grey trace shows the daily mean discharge (right y-axis) during the experiment. The average annual mean discharge at the nearby upstream gauge Heimborn is indicated by the grey horizontal line.

**Table 1**
Mean values (± SD) of the response variables in the impact stretch and the reference stretch during the dissimilarity phase and the similarity phase. Midstream: sampling sites limited to the middle of the river. Spring: sampling dates between May and mid-June. Sampling size (n) refers to the number of sampling dates.

| | Dissimilarity phase | | | Similarity phase | | |
|---|---|---|---|---|---|---|
| Response variable | Impact | Reference | n | Impact | Reference | n |
| DO [mg $L^{-1}$] | | | | | | |
| 8 cm | 3.91 ± 1.43 | 3.57 ± 1.34 | 12 | 3.43 ± 0.41 | 2.25 ± 0.49 | 19 |
| 8 cm, midstream | 3.61 ± 1.44 | 3.72 ± 1.39 | 12 | 4.19 ± 1.21 | 2.36 ± 0.48 | 19 |
| 8 cm, midstream, spring | 3.05 ± 0.64 | 3.40 ± 0.64 | 4 | 5.34 ± 0.91 | 2.69 ± 0.57 | 7 |
| 13 cm | 3.01 ± 1.25 | 2.48 ± 0.89 | 12 | 2.37 ± 0.54 | 1.84 ± 0.35 | 19 |
| 23 cm | 2.57 ± 1.05 | 1.90 ± ß.47 | 12 | 2.03 ± 0.75 | 1.62 ± 0.31 | 19 |
| 33 cm | 2.19 ± 0.74 | 1.87 ± 0.64 | 12 | 1.77 ± 0.37 | 1.56 ± 0.30 | 19 |
| Vertical flux [m $day^{-1}$] | 0.093 ± 0.017 | 0.099 ± 0.087 | 20 | 0.101 ± 0.011 | 0.085 ± 0.007 | 37 |
| Chl *a* (riffle) [μg $cm^{-2}$] | 14.0 ± 10.1 | 17.4 ± 10.7 | 17 | 22.5 ± 12.0 | 24.9 ± 12.4 | 20 |
| Chl *a* (pool) [μg $cm^{-2}$] | 13.3 ± 10.4 | 13.8 ± 9.3 | 17 | 10.4 ± 7.0 | 16.3 ± 13.3 | 20 |
| AFDM (riffle) [mg $cm^{-2}$] | 1.59 ± 0.99 | 2.02 ± 1.04 | 18 | 2.08 ± 0.70 | 2.62 ± 1.06 | 20 |
| AFDM (pool) [mg $cm^{-2}$] | 1.41 ± 0.92 | 1.40 ± 0.48 | 17 | 1.66 ± 1.00 | 1.83 ± 0.83 | 20 |

of eutrophication (Benndorf, 1990; Hansson et al., 1998). However, whether top-down control of the food web might be similarly used to mitigate eutrophication effects in running waters is unknown. Our large-scale field experiment clearly showed the high potential of fish stock manipulation to mitigate the effects of eutrophication in running waters, similar to biomanipulation in lakes. In accordance with our hypothesis the enhancement of herbivorous and omnivorous fish stocks mitigated eutrophication effects, evidenced by the significantly higher oxygen availability and higher rate of vertical water exchange in the hyporheic zone at relatively large spatial and temporal scales. These results are in line with those of a short-term mesocosm experiment conducted in the same river, which showed the positive effects of herbivorous nase and omnivorous chub on oxygen availability in the hyporheic zone (Hübner et al., 2020).

In our study, the effects on oxygen availability were restricted to the uppermost horizon of the hyporheic zone. The upper 10 cm layer of the hyporheic zone contains the highest faunal density, biomass and species richness and is therefore biologically most important (Palmer, 1997; Williams and Hynes, 1974; Winkelmann et al., 2003). Consequently, improving oxygen availability in the upper part of the hyporheic zone is a crucial indicator of the effectiveness of any biomanipulation. In addition, the restriction of the experimental effects to the upper reaches strongly supports the conclusion that fish stock manipulation was responsible for the positive effects in the hyporheic zone. Benthic foraging by chub has a depth-limited impact on hyporheic oxygen availability because these fish primarily disturb the surface layer of a gravel bed, as has been demonstrated before (e.g. Pledger et al., 2017, 2016). The fact that the increase in hyporheic oxygen availability was most pronounced in the middle of the river, where large fish and the grazing scars of nase on coarse substrates were most frequently observed, further supports the conclusion that the effects observed in our study were mediated by fish. Rheophilic nase likely preferred the middle of the river because of its higher current velocity and water depth (Huber and Kirchhofer, 1998). Especially during low-flow conditions, the water depth at some of the sampling sites near the river margin was insufficient for large fish. However, the depth restriction of the effects can also be explained by physical factors. For example, fine particles, such as detached algal cells, usually accumulate directly beneath the coarse armour layer of the hyporheic zone, resulting in the development of a relatively thin clogging layer during low-discharge periods (Brunke, 1999; Schälchli, 1992). Consequently, an abrupt reduction of particle input might have resulted in the same effects. Nonetheless, the occurrence of an abrupt change only in the impact stretch and at exactly the time of the experimental manipulation is not only very unlikely, it is also not supported by our data on sediment accumulation and fine sediment content.

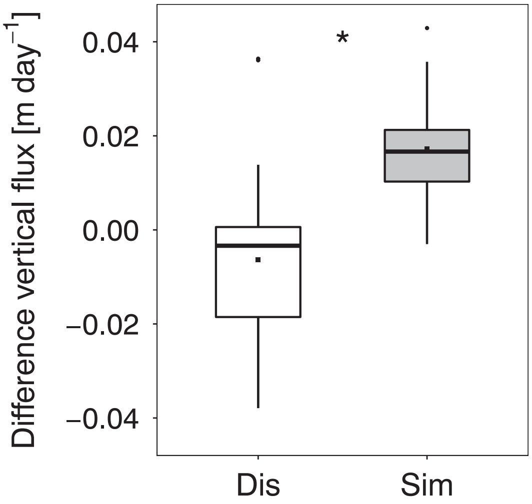


**Fig. 7.** Differences in the vertical flux of water in the hyporheic zone (5–10 cm depth) between the impact and reference stretches during the dissimilarity phase (Dis; $n = 20$, weekly mean values between April – August 2017) and the similarity phase (Sim; $n = 37$, weekly mean values between April – October 2018 and between April – July 2019). Boxes: 75% and 25%, whiskers: 95% and 5%, squares: mean differences, dots: outliers. *Significant ($p < 0.05$).

The extent of external clogging can be affected by grazers via a reduction of periphyton biomass and via declogging processes such as bioturbation by chub. The amount of decaying algal biomass in the hyporheic zone (i.e. the extent of internal clogging) can be affected by reducing periphyton directly (nase) and by facilitating the removal of decaying biomass via bioturbation (chub). Consequently, a combined reduction in external and internal clogging as well as the biological oxygen demand would explain the significant positive effects on hyporheic oxygen availability observed in our study despite only small reduction in periphyton biomass following fish stock enhancement.

This line of thought is further supported by our observation of significant positive effects of fish stock enhancement on vertical water flux in the upper layer of the hyporheic zone. This finding suggests that benthic foraging by chub disturbed the riverbed, which in turn reduced clogging in the upper layer of the hyporheic zone. Support for this explanation comes from an earlier mesocosm experiment, in which the presence of chub alone positively affected hyporheic oxygen availability even though it did not reduce periphyton biomass (Hübner et al., 2020). Moreover, evidence for bioturbation in natural riverbeds also comes from a previous field experiment in which chub and other benthic-feeding fish were shown to increase the mobility of sediments and thereby cause substrate coarsening (Pledger et al., 2017).

However, the apparent lack of a strong reduction of periphyton biomass might also be explained by the high spatial and temporal variability of periphyton biomass on the riverbed, which might have prevented the detection of grazing effects on a large scale (Ibisch et al., 2009). Compared to periphyton biomass, the extent of biological clogging in the hyporheic zone can be expected to change more slowly, because organic

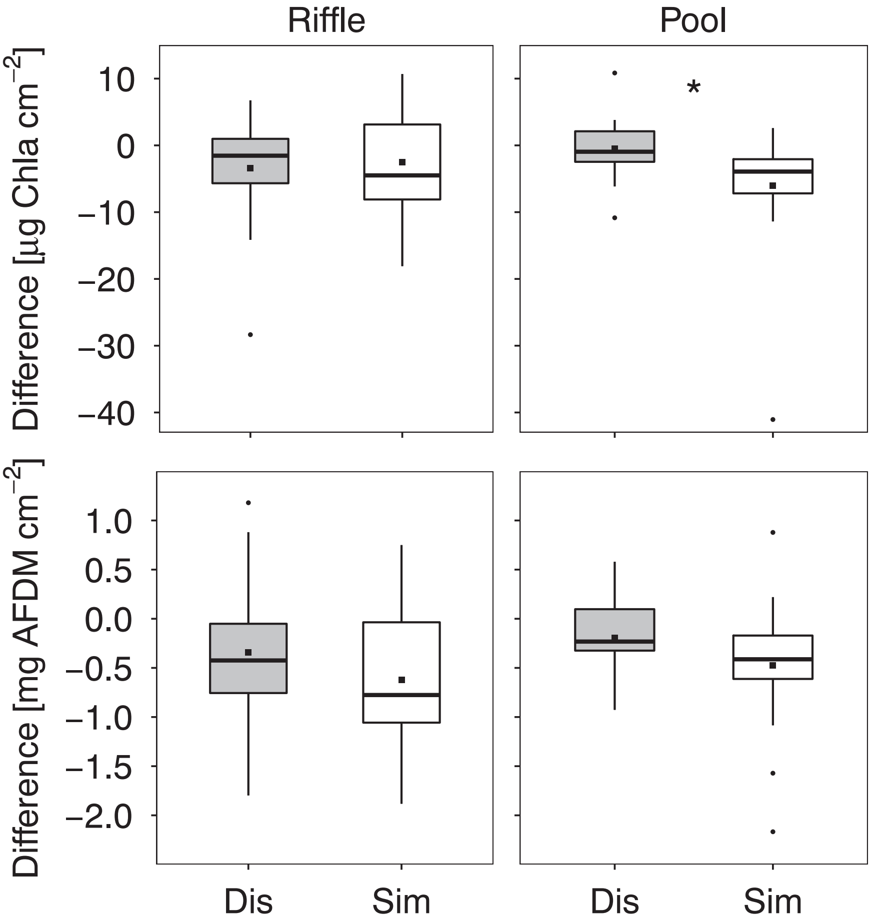


**Fig. 8.** Differences in periphyton biomass between the impact and reference stretches during the dissimilarity phase (Dis; $n \geq 17$) and the similarity phase (Sim; $n = 20$) at the riffle and the pool site. Upper row: differences in chlorophyll *a* concentrations per area, bottom row: differences in ash-free dry mass per area. Boxes: 75% and 25%, whiskers: 95% and 5%, squares: mean differences, dots: outliers. *Significant ($p < 0.05$).

matter accumulates in the hyporheic zone during low discharge and is only flushed out by the next flood (Brunke, 1999). This temporal and spatial integration may have facilitated the observation of the effects on hyporheic oxygen availability due to a much lower variability of hyporheic DO compared to periphyton biomass. Due to the methodological constraints inherent to any large-scale field experiment, we were unable to distinguish between the different mechanisms.

Nevertheless, our data showed a reduction of periphyton biomass at the pool site, which was likely caused by nase, because grazing effects of nase and smaller grazing fish species have been observed before (e.g. Hübner et al., 2020; Power et al., 1985). However, enhancing the density of large omnivorous chub might also have reduced periphyton biomass due to the indirect promotion of grazing by benthic invertebrates as a consequence of reduced predation by highly abundant small zoobenthivorous fish. The results of an earlier small-scale experiment conducted in the reference stretch of the same river indeed indicated a strong impact of the cascading effects of zoobenthivorous fish on periphyton biomass (Gerke et al., 2018). Indirect top-down control of periphyton through a three-level trophic cascade was also observed on a large scale under near-natural conditions (Winkelmann et al., 2014). In mesocosm experiments in a Northern California river, omnivorous fish were found to induce a four-level trophic cascade, down to periphyton, by reducing the abundance of primary predators and therefore predation pressure on grazing invertebrates (Power, 1990; Wootton and Power, 1993).

Although fish stock manipulation led to a relative improvement in hyporheic oxygen availability and vertical water flux, the absolute oxygen concentrations and vertical fluxes of water in the hyporheic zone at the experimental site were still rather low, especially under the prolonged low-flow conditions that occurred during the dry summer of 2018. The estimated vertical fluxes of water were at the lower end of the range determined in a similar gravel-bed river (0.11–1.08 m day$^{-1}$; Ingendahl et al., 2009). Low vertical water fluxes strongly indicate that the sediment permeability of a riverbed is affected not only by biological clogging due to eutrophication but also by physical clogging with inorganic fine sediments. The relatively high mean proportions of clay and silt-sized particles in our freeze-core samples are consistent with this observation. For comparison, they were close to the proportions of clay and silt-sized particles in freeze-core samples taken from a site in the transition zone of hyporhithral to epipotamal of a Mongolian gravel-bed river that is impaired by physical clogging due to intense pasture use (Hartwig and Borchardt, 2015). In addition to fine sediment loads, physical clogging in the Nister could arise from a bedload deficit resulting from erosion-control structures, such as bank reinforcements, in the upper reaches of the catchment. Consequently, the overall extent of physical clogging will not be considerably reduced by fish stock enhancement alone.

Furthermore, given the seasonal trend in both experimental stretches of declining hyporheic oxygen concentrations from spring until the end of the summer, it seems that the top-down effects of fish cannot avert but only attenuate or decelerate biological clogging. Consequently, in eutrophic rivers, mitigation measures limited to fish stock enhancement will not ensure good habitat quality for sensitive organisms, such as freshwater mussels, that require the sufficient, long-term availability of oxygen in the hyporheic zone (Geist and Auerswald, 2007). The measures could, however, support biodiversity conservation by enhancing habitat conditions during sensitive periods. In Central European rivers, the eggs and larvae of different spring-spawning fish, such as the salmonid grayling (*Thymallus thymallus*) and the cypriniform common nase, develop over a period of only a few weeks (Hübner et al., 2009; Kamler et al., 1998). During this intra-gravel period, they require a permeable and well-oxygenated hyporheic zone (salmonids: e.g. Malcolm et al., 2003; nase: e.g. Nagel et al., 2020). The positive effects of biomanipulation in our experiment were most pronounced during spring. Accordingly, the enhancement of herbivorous and omnivorous fish stocks may substantially improve habitat quality for the eggs and larvae of spring-spawning fish such as grayling and nase, especially when conducted in combination with the bioturbation resulting from spawning activity (Gutmann Roberts et al., 2020).

The relatively large spatial and temporal scales of the field experiment were a major strength of this study but also resulted in several of the difficulties common to field studies. For example, large fish could move freely within the long (> 500 m) river stretches but they could also move out of the experimental stretches when the dynamic fish weirs became submerged during high-flow conditions. This naturally impaired an exact adjustment of the fish stocks in both experimental stretches throughout the 4-year experiment. However, because we observed that both fish species are closely bound to their home ranges in the river, variability in fish densities is not expected to weaken the conclusions drawn from the experimental results. Furthermore, two experimental stretches in the field can never be identical. The main difference between the stretches in our study was that the total surface area of the river channel in the impact stretch was approximately 36% larger than in the reference stretch, mainly due to a wide shallow section in the upper part of the impact stretch. Because we estimated the fish stocks based on the length of the stretches during field work, fish density and biomass per area were similar but not identical in the reference and impact stretches during the similarity phase. However, the shallow upper part of the impact stretch was hardly used as a habitat by large fish, especially during the low-flow conditions in summer, because shallow non-turbulent areas have a higher predation risk and are thus usually avoided by adult fish (Allouche and Gaudin, 2001; Huber and Kirchhofer, 1998). The sampling sites in both experimental stretches included suitable resting and feeding habitats for large nase and chub, with a variety of depth and flow conditions as well as favourable structures such as tree roots and overhanging vegetation. It was therefore likely that the area of usable habitat for large fish and therefore the real distribution of fish densities during the similarity phase were similar in the two experimental stretches. However, adequate enhancement of fish stocks under non-experimental conditions, as would be necessary when biomanipulation is used as management tool, will no doubt be even more difficult than was the case under the conditions of our field study. As re-establishing large self-sustaining populations of the rheophilic specialist species common nase in rivers where population densities have strongly declined over the past decades might pose a major challenge in the establishment of biomanipulation as a management tool in running waters, the feasibility of this approach needs to be further investigated.

We expected positive effects of fish stocking on benthic invertebrate grazers due to a reduction in the abundance of small zoobenthivorous fish by the large omnivorous chub. Nevertheless, high densities of herbivorous and omnivorous fish may also exert negative effects on the benthic invertebrate community due to intense bioturbation or consumption of small benthic grazers inhabiting periphyton such as chironomids. In fact, grazing and detritivorous fishes have been shown to reduce the abundance of benthic invertebrate taxa in a tropical stream, either through resource depression, bioturbation or intimidation by fish (Flecker, 1992). However, in a previous mesocosm experiment, the presence of nase did not affect benthic invertebrates and the presence of chub positively affected benthic invertebrate biomass at fish densities that were even higher than in our reach-scale experiment (Hübner et al., 2020). Nevertheless, future studies should also examine potential ecosystem-scale effects of fish stock enhancement on the benthic invertebrate community.

## 5. Conclusion

Our study is the first to demonstrate that the use of biomanipulation, via the enhancement of large herbivorous and omnivorous fish stocks, has the potential to mitigate the effects of eutrophication in medium-sized European rivers. High fish densities increased hyporheic oxygen availability and can thus be expected to substantially improve habitat quality, especially during the short intra-gravel period of spring-spawning fish species. However, the top-down effects of fish on periphyton biomass were rather small and their effects on the hyporheic zone did not seem to be strong enough to reduce the extent of physical clogging or to avoid biological clogging during prolonged low-flow conditions. While biomanipulation can contribute to mitigating the effects of eutrophication, sustainable restoration of the hyporheic zone in eutrophic rivers, and in turn the preservation of biodiversity, might only be achieved when it is used in combination with a reduction of nutrient and fine sediment inputs.

## CRediT authorship contribution statement

**Madlen Gerke:** Investigation, Formal analysis, Visualization, Writing - original draft. **Dirk Hübner:** Funding acquisition, Resources, Investigation, Writing - review & editing. **Jörg Schneider:** Funding acquisition, Resources, Investigation, Writing - review & editing. **Carola Winkelmann:** Conceptualization, Methodology, Funding acquisition, Project administration, Supervision, Resources, Writing - review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

We especially wish to thank Christian Sodemann and Manfred Fetthauer for their technical advice and valuable support throughout the 4-year experiment. We also thank the colleagues, students and volunteers of the local river protection association ARGE Nister e.V. who helped with field work and/or laboratory analyses, especially Michael Götten, Theresa Graf, Roman Fricke, Timo Seufert, Christoph Mentzel, Barbara Nuyken and Ulli Bange. We are also grateful to Wendy Ran for linguistic support.

## Funding

This work was financially supported by the Federal Office for Agriculture and Food of Germany [grant numbers 2813BM011, 2818BM084].

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.142547.

## References

Allouche, S., Gaudin, P., 2001. Effects of avian predation threat, water flow and cover on growth and habitat use by chub, *Leuciscus cephalus*, in an experimental stream. Oikos 94, 481–492. https://doi.org/10.1034/j.1600-0706.2001.940310.x.

Balestrieri, A., Prigioni, C., Remonti, L., Sgrosso, S., Priore, G., 2006. Feeding ecology of *Leuciscus cephalus* and *Rutilus rubilio* in southern Italy. Italian Journal of Zoology 73, 129–135. https://doi.org/10.1080/11250000600679561.

Benndorf, J., 1990. Conditions for effective biomanipulation; conclusions derived from whole-lake experiments in Europe. Hydrobiologia 200 (201), 187–203.

Bowes, M.J., Ings, N.L., McCall, S.J., Warwick, A., Barrett, C., Wickham, H.D., Harman, S.A., Armstrong, L.K., Scarlett, P.M., Roberts, C., Lehmann, K., Singer, A.C., 2012. Nutrient and light limitation of periphyton in the river Thames: implications for catchment management. Sci. Total Environ. 434, 201–212. https://doi.org/10.1016/j.scitotenv.2011.09.082.

Brunke, M., 1999. Colmation and depth filtration within streambeds: retention of particles in hyporheic interstices. Int. Rev. Hydrobiol. 84, 99–117. https://doi.org/10.1002/iroh.199900014.

Burrell, T.K., O'Brien, J.M., Graham, S.E., Simon, K.S., Harding, J.S., McIntosh, A.R., 2014. Riparian shading mitigates stream eutrophication in agricultural catchments. Freshwater Science 33, 73–84. https://doi.org/10.1086/674180.

Carpenter, S.R., Frost, T.M., Heisey, D., Kratz, T.K., 1989. Randomized intervention analysis and the interpretation of whole-ecosystem experiments. Ecology 70, 1142–1152. https://doi.org/10.2307/1941382.

Corse, E., Costedoat, C., Chappaz, R., Pech, N., Martin, J.-F., Gilles, A., 2010. A PCR-based method for diet analysis in freshwater organisms using 18S rDNA barcoding on faeces. Mol. Ecol. Resour. 10, 96–108. https://doi.org/10.1111/j.1755-0998.2009.02795.x.

Dahm, V., Hering, D., Nemitz, D., Graf, W., Schmidt-Kloiber, A., Leitner, P., Melcher, A., Feld, C.K., 2013. Effects of physico-chemistry, land use and hydromorphology on three riverine organism groups: a comparative analysis with monitoring data from Germany and Austria. Hydrobiologia 704, 389–415. https://doi.org/10.1007/s10750-012-1431-3.

Dodds, W.K., Smith, V.H., Lohman, K., 2002. Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. Can. J. Fish. Aquat. Sci. 59, 865–874. https://doi.org/10.1139/f02-063.

Dodds, W.K., Smith, V.H., Lohman, K., 2006. Erratum: nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. Can. J. Fish. Aquat. Sci. 63, 1190–1191. https://doi.org/10.1139/f06-040.

Feminella, J.W., Hawkins, C.P., 1995. Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. J. N. Am. Benthol. Soc. 14, 465–509. https://doi.org/10.2307/1467536.

Flecker, A.S., 1992. Fish trophic guilds and the stucture of a tropical stream: weak direct vs. strong indirect effects. Ecology 73, 927–940. https://doi.org/10.2307/1940169.

Freyhof, J., 1995. Feeding traces as an indicator of habitat choice in Nase, *Chondrostoma nasus*. Folia Zool. 44, 57–61.

Geist, J., Auerswald, K., 2007. Physicochemical stream bed characteristics and recruitment of the freshwater pearl mussel (*Margaritifera margaritifera*). Freshw. Biol. 52, 2299–2316. https://doi.org/10.1111/j.1365-2427.2007.01812.x.

Gelwick, F.P., Matthews, W.J., 1992. Effects of an algivorous minnow on temperate stream ecosystem properties. Ecology 73, 1630–1645. https://doi.org/10.2307/1940016.

Gerke, M., Cob Chaves, D., Richter, M., Mewes, D., Schneider, J., Hübner, D., Winkelmann, C., 2018. Benthic grazing in a eutrophic river: cascading effects of zoobenthivorous fish mask direct effects of herbivorous fish. PeerJ 6, e4381. https://doi.org/10.7717/peerj.4381.

Ghermandi, A., Vandenberghe, V., Benedetti, L., Bauwens, W., Vanrolleghem, P.A., 2009. Model-based assessment of shading effect by riparian vegetation on river water quality. Ecol. Eng. 35, 92–104. https://doi.org/10.1016/j.ecoleng.2008.09.014.

Gil, M.A., Jiao, J., Osenberg, C.W., 2016. Enrichment scale determines herbivore control of primary producers. Oecologia 180, 833–840. https://doi.org/10.1007/s00442-015-3505-1.

Gordon, R.P., Lautz, L.K., Briggs, M.A., McKenzie, J.M., 2012. Automated calculation of vertical pore-water flux from field temperature time series using the VFLUX method and computer program. J. Hydrol. 420–421, 142–158. https://doi.org/10.1016/j.jhydrol.2011.11.053.

Gutmann Roberts, C., Bašić, T., Britton, J.R., Rice, S., Pledger, A.G., 2020. Quantifying the habitat and zoogeomorphic capabilities of spawning European barbel *Barbus barbus*, a lithophilous cyprinid. River Res. Appl. 36, 259–279. https://doi.org/10.1002/rra.3573.

Hansson, L.-A., Annadotter, H., Bergman, E., Hamrin, S.F., Jeppesen, E., Kairesalo, T., Luokkanen, E., Nilsson, P.-Å., Søndergaard, M., Strand, J., 1998. Biomanipulation as an application of food-chain theory: constraints, synthesis, and recommendations for temperate lakes. Ecosystems 1, 558–574.

Hartwig, M., Borchardt, D., 2015. Alteration of key hyporheic functions through biological and physical clogging along a nutrient and fine-sediment gradient. Ecohydrology 8, 961–975. https://doi.org/10.1002/eco.1571.

Hellawell, J.M., 1971. The autecology of the chub, *Squalius cephalus* (L.), of the River Lugg and the Afon Llynfi. Freshw. Biol. 1, 369–387. https://doi.org/10.1111/j.1365-2427.1971.tb01569.x.

Hering, D., Johnson, R.K., Kramm, S., Schmutz, S., Szoszkiewicz, K., Verdonschot, P.F.M., 2006. Assessment of European streams with diatoms, macrophytes, macroinvertebrates and fish: a comparative metric-based analysis of organism response to stress. Freshw. Biol. 51, 1757–1785. https://doi.org/10.1111/j.1365-2427.2006.01610.x.

Hillebrand, H., 2009. Meta-analysis of grazer control of periphyton biomass across aquatic ecosystems. J. Phycol. 45, 798–806. https://doi.org/10.1111/j.1529-8817.2009.00702.x.

Huber, M., Kirchhofer, A., 1998. Radio telemetry as a tool to study habitat use of nase (*Chondrostoma nasus* L.) in medium-sized rivers. Hydrobiologia 371, 309–319. https://doi.org/10.1023/A:1017005523302.

Hübner, D., Borchardt, D., Fischer, J., 2009. Cascading effects of eutrophication on intragravel life stages of European Grayling (*Thymallus thymallus* L.). fundamental and applied limnology. Advances in Applied Limnology 61, 205–224.

Hübner, D., Gerke, M., Fricke, R., Schneider, J., Winkelmann, C., 2020. Cypriniform fish in running waters reduce hyporheic oxygen depletion in a eutrophic river. Freshw. Biol. 00, 1–11. https://doi.org/10.1111/fwb.13517.

Humpesch, U.H., Niederreiter, R., 1993. Freeze-core method for sampling the vertical distribution of the macrozoobenthos in the main channel of a large deep river, the River Danube at river kilometre 1889. Large Rivers, 87–90 https://doi.org/10.1127/lr/9/1993/87.

Ibisch, R.B., Borchardt, D., 2002. Effects of periphyton biomass and suspended solids on river bed permeability and hyporheic oxygen balances. SIL Proceedings 1922-2010 (28), 1875–1879. https://doi.org/10.1080/03680770.2001.11901954.

Ibisch, R.B., Seydell, I., Borchardt, D., 2009. Influence of periphyton biomass dynamics on biological colmation processes in the hyporheic zone of a gravel bed river (River Lahn, Germany). Fundamental and Applied Limnology, Advances in Applied Limnology 87–104.

Ingendahl, D., Borchardt, D., Saenger, N., Reichert, P., 2009. Vertical hydraulic exchange and the contribution of hyporheic community respiration to whole ecosystem respiration in the River Lahn (Germany). Aquat. Sci. 71, 399–410. https://doi.org/10.1007/s00027-009-0116-0.

Irvine, D.J., Lautz, L.K., Briggs, M.A., Gordon, R.P., McKenzie, J.M., 2015. Experimental evaluation of the applicability of phase, amplitude, and combined methods to determine water flux and thermal diffusivity from temperature time series using VFLUX 2. J. Hydrol. 531, 728–737. https://doi.org/10.1016/j.jhydrol.2015.10.054.

Jarvie, H.P., Sharpley, A.N., Withers, P.J.A., Scott, J.T., Haggard, B.E., Neal, C., 2013. Phosphorus mitigation to control river eutrophication: murky waters, inconvenient truths, and "postnormal" science. J. Environ. Qual. 42, 295–304. https://doi.org/10.2134/jeq2012.0085.

Kamler, E., Keckei, H., Bauer-Nemeschkal, E., 1998. Temperature-induced changes of survival, development and yolk partitioning in *Chondrostoma nasus*. J. Fish Biol. 53, 658–682. https://doi.org/10.1111/j.1095-8649.1998.tb01009.x.

Keckeis, H., Bauer-Nemeschkal, E., Kamler, E., 1996. Effects of reduced oxygen level on the mortality and hatching rate of *Chondrostoma nasus* embryos. J. Fish Biol. 49, 430–440. https://doi.org/10.1111/j.1095-8649.1996.tb00039.x.

Keery, J., Binley, A., Crook, N., Smith, J.W.N., 2007. Temporal and spatial variability of groundwater–surface water fluxes: development and application of an analytical method using temperature time series. J. Hydrol. 336, 1–16. https://doi.org/10.1016/j.jhydrol.2006.12.003.

Lenk, M., Saenger, N., Traebing, K., Killich, F., Fischer, J., Borchardt, D., 1999. Die multi-level-interstitialsonde: Methode zur tiefenorientierten Untersuchung von Gewässersedimenten. DVWK Schriften 128, 150–157.

Lusk, S., 1967. Population dynamics of *Chondrostoma nasus* (Linnaeus, 1758) in the Rokytná river. Acta Sc. Nat. Brno 1, 473–522.

Lyche Solheim, A., Globevnik, L., Austnes, K., Kristensen, P., Moe, S.J., Persson, J., Phillips, G., Poikane, S., van de Bund, W., Birk, S., 2019. A new broad typology for rivers and lakes in Europe: Development and application for large-scale environmental assessments. Sci. Total Environ. 697, 134043. https://doi.org/10.1016/j.scitotenv.2019.134043.

Malcolm, I.A., Youngson, A.F., Soulsby, C., 2003. Survival of salmonid eggs in a degraded gravel-bed stream: effects of groundwater–surface water interactions. River Res. Appl. 19, 303–316. https://doi.org/10.1002/rra.706.

Mann, R.H.K., 1976. Observations on the age, growth, reproduction and food of the chub *Squalius cephalus* (L.) in the River Stour, Dorset. J. Fish Biol. 8, 265–288. https://doi.org/10.1111/j.1095-8649.1976.tb03950.x.

McDowell, R.W., Noble, A., Pletnyakov, P., Haggard, B.E., Mosley, L.M., 2020. Global mapping of freshwater nutrient enrichment and periphyton growth potential. Sci. Rep. 10, 3568. https://doi.org/10.1038/s41598-020-60279-w.

Mewes, D., Spielvogel, S., Winkelmann, C., 2017. RNA/DNA ratio as a growth indicator of stream periphyton. Freshw. Biol. 62, 807–818. https://doi.org/10.1111/fwb.12903.

Mühlbauer, M., Traxler, E., Zitek, A., Schmutz, S., 2003. Ein hochwassersicheres Fischwehr zur Untersuchung der Fischwanderung in kleinen bis mittelgroßen Flüssen. Österreichs Fischerei 56, 98–102.

Nagel, C., Pander, J., Mueller, M., Geist, J., 2020. Substrate composition determines emergence success and development of European nase larvae (*Chondrostoma nasus* L.). Ecol. Freshw. Fish 29, 121–131. https://doi.org/10.1111/eff.12500.

Palmer, M.A., 1997. Biodiversity and ecosystem processes in freshwater sediments. Ambio 26, 571–577.

Peňáz, M., 1996. *Chondrostoma nasus* - its reproduction strategy and possible reasons for a widely observed population decline - a review. In: Kirchhofer, A., Hefti, D. (Eds.), Conservation of Endangered Freshwater Fish in Europe. Birkhäuser Basel, Basel, pp. 279–285 https://doi.org/10.1007/978-3-0348-9014-4_27.

Pledger, A.G., Rice, S.P., Millett, J., 2016. Bed disturbance via foraging fish increases bedload transport during subsequent high flows and is controlled by fish size and species. Geomorphology 253, 83–93. https://doi.org/10.1016/j.geomorph.2015.09.021.

Pledger, A., Rice, S., Millett, J., 2017. Foraging fish as zoogeomorphic agents: an assessment of fish impacts at patch, barform, and reach scales. Journal of Geophysical Research: Earth Surface 122, 2105–2123. https://doi.org/10.1002/2017J F004362.

Power, M.E., 1990. Effects of fish in river food webs. Science 250, 811–814. https://doi.org/10.1126/science.250.4982.811.

Power, M.E., Matthews, W.J., Stewart, A.J., 1985. Grazing minnows, piscivorous bass, and stream algae: dynamics of a strong interaction. Ecology 66, 1448–1456. https://doi.org/10.2307/1938007.

R Development Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reckendorfer, W., Keckeis, H., Tiitu, V., Winkler, G., Zornig, H., 2001. Diet shifts in 0+ nase, *Chondrostoma nasus*: size-specific differences and the effect of food. Arch. Hydrobiol. Suppl 13512, 425–440.

Ricker, W.E., 1975. Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada 191, 382.

Schälchli, U., 1992. The clogging of coarse gravel river beds by fine sediment. Hydrobiologia 235, 189–197. https://doi.org/10.1007/BF00026211.

SGD Nord, 2020. Hintergrund und Zielsetzungen zum Nisterprogramm. Local Environmental Agency, Rhineland-Palatinate, Germany. https://sgdnord.rlp.de/fileadmin/sgdnord/Wasser/Aktion_Blau_plus/Nisterprogramm/Hintergrundpapier_zum_Nisterprogramm.pdf. (Accessed 31 July 2020).

Smith, E.P., Orvos, D.R., Cairns Jr., J., 1993. Impact assessment using the before-after-control-impact (BACI) model: concerns and comments. Can. J. Fish. Aquat. Sci. 50, 627–637. https://doi.org/10.1139/f93-072.

Smith, V.H., Tilman, G.D., Nekola, J.C., 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ. Pollut. 100, 179–196. https://doi.org/10.1016/S0269-7491(99)00091-3.

Stewart, A.J., 1987. Responses of stream algae to grazing minnows and nutrients: a field test for interactions. Oecologia 72, 1–7.

Stewart-Oaten, A., Murdoch, W.W., Parker, K.R., 1986. Environmental impact assessment: “pseudoreplication” in time? Ecology 67, 929–940. https://doi.org/10.2307/1939815.

Vater, M., 1997. Age growth of the undermouth *Chondrostoma nasus* in the Slovak stretch of the Danube river. Biologia, Bratislava 52 653–651.

Williams, D.D., Hynes, H.B.N., 1974. The occurrence of benthos deep in the substratum of a stream. Freshw. Biol. 4, 233–256. https://doi.org/10.1111/j.1365-2427.1974.tb00094.x.

Winkelmann, C., Koop, J.H.E., Benndorf, J., 2003. Abiotic features and macroinvertebrate colonization of the hyporheic zones of two tributaries of the river Elbe (Germany). Limnologica 33, 112–121. https://doi.org/10.1016/S0075-9511(03)80040-3.

Winkelmann, C., Schneider, J., Mewes, D., Schmidt, S.I., Worischka, S., Hellmann, C., Benndorf, J., 2014. Top-down and bottom-up control of periphyton by benthivorous fish and light supply in two streams. Freshw. Biol. 59, 803–818. https://doi.org/10.1111/fwb.12305.

Wootton, J.T., Power, M.E., 1993. Productivity, consumers, and the structure of a river food chain. Proc. Natl. Acad. Sci. 90, 1384–1387. https://doi.org/10.1073/pnas.90.4.1384.