Conservation of freshwater fish biodiversity – a case study of the common nase

Chondrostoma nasus L.

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„In den kleinsten Dingen zeigt die Natur die allergrößten Wunder.“

Carl von Linné (1707 - 1778)

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Summary

Freshwater ecosystems are among the most threatened ecosystems worldwide, yet harbor a high level of biodiversity. The heavy anthropogenic alteration of freshwater ecosystems has led to an alarming loss of aquatic biodiversity in recent decades, especially in the group of freshwater fish. In addition to severe population declines and increasing species extinctions, the acute threat is also linked to a loss in genetic resources, habitat diversity, and important functional processes in these ecosystems. Therefore, in order to develop effective conservation strategies for freshwater fish and their habitats, it is essential to pursue holistic conservation strategies that consider all levels of biodiversity organisation and the full life cycle of a fish. Similarly, it is important to have an accurate knowledge of the ecology and life stage-specific habitat requirements of the targeted species, especially concerning the particular sensitive egg and larval stages. Consequently, the main objectives of this thesis were (1.) to characterise the different life stages and habitat requirements of the endangered target species common nase (*Chondrostoma nasus* L.), (2.) to systematically investigate different measures for the conservation of this species and (3.) to evaluate classical and modern techniques for monitoring the applied conservation measures. To achieve these objectives, six case studies were conducted, including laboratory experiments and field trials in natural habitats, involving a wide range of conservation and monitoring techniques.

Supportive breeding activities play a significant role in conservation of freshwater fish, even for less economically relevant species such as *Chondrostoma nasus*. However, the effect of these initiatives on the genetic constitution of the offspring has rarely been evaluated. Chapter 4 of this dissertation demonstrated that the selection of a very limited number of spawners has detrimental genetic effects on offspring by reducing genetic diversity and increasing inbreeding parameters. Based on these results, several measures have been developed in this thesis to better maintain the genetic integrity of *Chondrostoma nasus* in supportive breeding programs, some of which can be applied to other species. Improvements can be realised through an adapted selection of females and males and alternative means of egg collection. This study has also shown that an accompanying genetic monitoring should be a core component in supportive breeding activities.

However, maintaining intraspecific diversity in conservation management is not only important at the genetic but also at the phenotypic level. The case study in Chapter 5, in which egg material from different populations of *Chondrostoma nasus* was examined at high resolution using scanning electron microscopy (SEM) in the laboratory, revealed clear intraspecific differences in egg properties between the populations studied. These differences may indicate local adaptation to the respective spawning grounds. Moreover, it could be shown that the developed assessment matrix for SEM imaging of fish eggs, combined with the use of multivariate statistical analysis, is a useful tool for identifying intraspecific variability in fish populations.

Another study conducted under controlled laboratory conditions (Chapter 6), revealed further insights into the ecology of *Chondrostma nasus* by showing that increased levels of fine sediment in the spawning substrate negatively affect hatching and emergence success, as well as larval growth. Such evidence was previously only known for species of the salmonid family and was not expected for substrate-spawning cyprinids such as *Chondrostoma nasus*. To validate these results under realistic conditions in natural habitats, a field experiment was conducted in which two known spawning sites of *Chondrostoma nasus* were divided into equal halves and subsequently the substrate of one half each was restored (Chapter 7). As a result, a significant reduction in the fine sediment content (-70%) was achieved. During spawning, *Chondrostoma nasus* clearly preferred the restored halves of the spawning grounds to the unrestored ones, and eggs seeped deeper into the interstitial zone in the restored substrate. In addition, hatched larvae remained in the sheltering substrate interstices for up to two weeks before emergence. Both studies highlight the previously overlooked importance of substrate quality and vertical connectivity at spawning grounds of *Chondrostoma nasus* and provide crucial information for the restoration of these key habitats.

In the life cycle of *Chondrostoma nasus* emergence of larvae from the spawning substrate is followed by downstream drift, which is an elementary process in the search for suitable foraging habitats, yet is often severely disturbed in regulated rivers. An extensive field study in Chapter 8 examined spatio-temporal drift patterns of egg and larval stages in the large and heavily regulated alpine River Inn and a newly constructed nature-like bypass system in this river. This study revealed a distinct seasonal chronology of larval drift of the fish species community of this river, including *Chondrostoma nasus*, which was the most abundant

species. In addition, a clear shift towards nocturnal drift activity was observed in almost all fish species. These results can be taken as an initial basis for directing discharge and turbine management in hydropower dominated alpine streams in a more "fish larvae friendly" way during periods of peak drift activity, e.g. by strengthening bypass corridors. In addition, taxonomic composition of drift samples showed that nature-like bypass systems can provide functional spawning sites for *Chondrostoma nasus* and other endangered gravel-spawning species, such as European grayling (*Thymallus thymallus* L.). This finding clearly underpins the ecological value of nature-like fishways, in addition to solely restoring longitudinal connectivity. Finally, an approach for a rapid and inexpensive genetic species identification of fish larvae was developed that can be transferred to the taxonomic analysis of the larval fish community in other streams. This is of particular importance as it is increasingly recognised in the scientific community that fish larvae identification solely based on morphometric criteria is often not precise.

Juvenile habitats for riverine fish are mostly located in low-flow areas in the floodplain, yet these habitats have largely vanished as a result of the straightening and stabilisation of riverbanks. Another field study, presented in Chapter 9, evaluated the effect of bank habitat restoration and associated improvement of lateral connectivity in the highly modified Alpine River Inn by comparing three bank habitat types, including heavily stabilised and restored bank zones, and monitoring the use of these habitats by the fish species community. The results of this study demonstrated that even a relatively simple and inexpensive restoration measure such as the removal of bank stabilising structures can be successful in creating habitat diversity and thereby providing important habitats for juvenile stages of riverine fish species, including *Chondrostoma nasus*. This study also provided important insights for future restoration of bank habitats in rivers with highly variable flow, which is particularly important in light of the predicted impacts of climate change.

Using *Chondrostoma nasus* as a role model, the results of this thesis demonstrate that effective conservation management of freshwater fish biodiversity benefits from a holistic approach, which integrates the full life cycle of a species, all levels of biodiversity organisation, and the multidimensionality of lotic ecosystems. To this end, it is indispensable to have an accurate ecological knowledge of the targeted species. As the results of this thesis reveal, such knowledge can still be enhanced by important new findings, even in relatively well-studied

species such as *Chondrostoma nasus*. In addition, effective conservation management must be aware of the strengths and pitfalls of applied conservation measures while it is equally important to evaluate their success by using the whole toolkit of conventional and advanced monitoring techniques. Owing from the broad set of conservation tools and monitoring techniques evaluated in this thesis, specific recommendations can be derived from the results obtained, supporting a comprehensive improvement of conservation efforts for *Chondrostoma nasus*. These include the maintenance of genetic integrity during supportive breeding programmes as well as the restoration of key habitats and the enhancement of habitat connectivity in vertical, longitudinal and lateral dimensions. These results are broadly transferable to other rheophilic riverine fish species with a similar ecology. Since most restoration measures applied in practice are still very restricted in their spatio-temporal extend, the general paradigm in freshwater biodiversity conservation should be shifted from a rather species-based approach towards a more process-based one, which allows for a much more comprehensive and sustainable scaling of conservation measures.

Zusammenfassung

Fließgewässer gehören zu den am stärksten bedrohten Ökosystemen weltweit, beherbergen jedoch ein hohes Maß an Biodiversität. Die starke anthropogene Nutzung von Fließgewässern hat in den letzten Jahrzehnten zu einem alarmierenden Verlust von aquatischer Biodiversität geführt, insbesondere in der Gruppe der Süßwasserfische. Neben dem starken Populationsrückgang und zunehmenden Aussterben von Arten ist auch ein Rückgang der genetischen Ressourcen, der Lebensraumvielfalt und wichtiger funktioneller Prozesse in Fließgewässer-Ökosystemen sichtbar. Um wirksame Schutzstrategien für den Erhalt von Süßwasserfischen und ihren Lebensräumen zu entwickeln, ist es daher essentiell, ganzheitliche Ansätze zu verfolgen, die alle Ebenen der Biodiversität und den vollständigen Lebenszyklus eines Fisches berücksichtigen. Ebenso wichtig ist es, umfangreiche Kenntnis über die Ökologie und lebensstadienspezifische Habitatansprüche der Zielart zu haben, insbesondere der sehr sensiblen Ei- und Larvenstadien. Die übergeordneten Ziele dieser Dissertation waren daher, am Beispiel der bedrohten Modellart Nase (*Chondrostoma nasus* L.), (1.) die verschiedenen Lebensstadien und ihre Habitatbedürfnisse zu charakterisieren, (2.) systematisch verschiedene Werkzeuge zum Erhalt dieser Art zu untersuchen und (3.) klassische und moderne Techniken für das Monitoring der eingesetzten Schutzmaßnahmen zu evaluieren. Um diese Ziele zu erreichen, wurden sechs Fallstudien durchgeführt, die sowohl Laborexperimente als auch Feldversuche in natürlichen Habitaten umfassten und dabei ein breites Spektrum verschiedener Techniken einsetzten.

Nachzuchtmaßnahmen spielen zunehmend auch bei ökonomisch weniger relevanten Arten wie *Chondrostoma nasus* eine bedeutende Rolle beim Schutz und Wiederaufbau von Populationen, ihr Einfluss auf die genetische Konstitution der Nachkommen wird dabei jedoch nur selten evaluiert. So konnte Kapitel 4 dieser Dissertation zeigen, dass die Selektion von nur wenigen Elterntieren für Nachzuchtmaßnahmen messbare negative genetische Effekte auf die Nachkommen hat, da sie die genetische Diversität verringert und Inzucht-Parameter erhöht. Aufbauend auf diesen Ergebnissen wurden anschließend mehrere Maßnahmen entwickelt, um die genetische Integrität von *Chondrostoma nasus* im Rahmen von Nachzuchtprogrammen besser zu gewährleisten, die teilweise auch auf andere Arten übertragen werden können. Verbesserungen sind beispielsweise über eine angepasste Kreuzung von Rognern und Milchnern und alternative Möglichkeiten der Eigewinnung möglich.

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Der Erhalt intraspezifischer Diversität ist jedoch nicht nur auf genetischer, sondern auch auf phänotypischer Ebene ein wichtiger Faktor innerhalb eines ganzheitlichen Schutzmanagements. Eine Fallstudie (Kapitel 5), in der das Eimaterial verschiedener Populationen von *Chondrostoma nasus* im Labor mittels Rasterelektronenmikroskopie hochauflösend untersucht wurde, ergab klare intraspezifische Unterschiede in der Oberflächenstruktur der Eier der untersuchten Populationen. Diese Unterschiede können auf eine lokale Anpassung an die jeweiligen Laichplätze hindeuten. Zudem stellte sich heraus, dass die entwickelte Bewertungsmatrix für rasterelektronenmikroskopische Aufnahmen von Fischeiern in Kombination mit dem Einsatz multivariater, statistischer Analyseinstrumente ein nützliches Instrument zur Ermittlung intraspezifischer Variabilität von Fischpopulationen darstellt.

Weitere wichtige Erkenntnisse zur Ökologie von *Chondrostoma nasus* konnten im Rahmen einer weiteren Studie (Kapitel 6), die unter kontrollierten Laborbedingungen durchgeführt wurde, gewonnen werden. Hierbei wurde deutlich, dass erhöhte Anteile von Feinsediment im Laichsubstrat den Schlupf- und Emergenzerfolg sowie das Wachstum der Larven negativ beeinflussen. Ein solcher Befund war bisher nur für Arten aus der Familie der Salmoniden bekannt und wurde für kieslaichende Cypriniden wie *Chondrostoma nasus* in der internationalen Fachliteratur bisher nicht angenommen. Um diese Ergebnisse unter realistischen Bedingungen in natürlichen Habitaten von *Chondrostoma nasus* zu prüfen, wurde parallel ein Feldexperiment durchgeführt (Kapitel 7). Dabei wurden zwei bekannte Laichplätze in gleich große Hälften geteilt, wobei jeweils das Substrat einer Laichplatzhälfte anschließend restauriert wurde. In dem restaurierten Laichplatzareal konnte eine deutliche Reduktion des Feinsedimentanteils um 70% erreicht werden. Während des Laichens stellte sich heraus, dass *Chondrostoma nasus* bei der Abgabe der Eier die restaurierte Laichplatzhälfte gegenüber der nicht restaurierten deutlich bevorzugte und die Eier dort tiefer in das Kieslückensystem einsickerten. Ein weiteres wichtiges Ergebnis war, dass sich die Embryonen nach dem Schlupf bis zu zwei Wochen im schützenden Kieslückensystem aufhielten bevor sie als fertig entwickelte Larven emergierten. Sowohl Labor- als auch Feldstudie heben die bisher übersehene Bedeutung der Substratqualität und der vertikalen Vernetzung zwischen Kieslückensystem und Freiwasser auf den Laichplätzen von *Chondrostoma nasus* hervor und liefern so zentrale Informationen für die Renaturierung dieser Schlüsselhabitate.

Im Lebenszyklus von *Chondrostoma nasus* schließt sich nach der Emergenz aus dem Laichsubstrat die stromabwärts gerichtete Drift an. Sie stellt einen elementaren Prozess auf der Suche nach geeigneten Jungfischhabitaten dar, ist jedoch in regulierten Flüssen häufig empfindlich gestört. Im Rahmen einer umfangreichen Feldstudie in Kapitel 8 wurden zeitlich-räumliche Driftmuster der Ei- und Larvenstadien in dem stark regulierten Alpenfluss Inn und einem dort neu angelegten, naturnahen Umgehungsgewässer untersucht. Die Studie zeigte eine klare jahreszeitliche Chronologie der Larvendrift der dort vorhandenen Fischartengemeinschaft, einschließlich *Chondrostoma nasus*, die am häufigsten nachgewiesen wurde. Bei fast allen Fischarten wurde zudem eine deutliche Verschiebung hin zur nächtlichen Driftaktivität beobachtet. Diese Ergebnisse können als Grundlage dienen, um das Abfluss- und Turbinenmanagement in von Wasserkraft geprägten alpinen Fließgewässern in Zeiten hoher Driftaktivität zukünftig "fischlarvenfreundlich" zu gestalten, indem beispielsweise Driftkorridore wie Umgehungsgewässer in dieser Zeit stärker mit Wasser beaufschlagt werden. Die taxonomische Zusammensetzung der Driftproben zeigte außerdem, dass naturnahe Umgehungsgewässer funktionale Laichplätze für *Chondrostoma nasus*, aber auch für andere gefährdete kieslaichende Arten, wie der Äsche (*Thymallus thymallus* L.), bereitstellen können. Neben dem Aspekt der reinen Wiederherstellung der longitudinalen Vernetzung unterstreichen die Resultate so deutlich den ökologischen Zusatznutzen naturnah ausgestalteter Fischwanderhilfen. Abschließend wurde im Rahmen der Studie ein Verfahren entwickelt, das eine relativ schnelle und kostengünstige genetische Artbestimmung von Fischlarven ermöglicht und auf die Fischartengemeinschaft in anderen Fließgewässern übertragen werden kann. Da sich in wissenschaftlichen Publikationen zunehmend die Erkenntnis durchsetzt, dass eine rein morphometrische Identifizierung von Fischlarven oft nicht präzise genug ist, sind solche methodischen Entwicklungen besonders bedeutsam.

Jungfischhabitate von Flussfischen befinden sich meist in strömungsberuhigten Uferzonen. Mittlerweile sind diese jedoch durch die Begradigung und Befestigung der Flussufer weitgehend verschwunden. Eine weitere Feldstudie, die in Kapitel 9 vorgestellt wird, untersuchte deshalb die Effekte einer Renaturierung von Uferhabitaten und der damit verbundenen Verbesserung der lateralen Konnektivität im erheblich regulierten Alpenfluss Inn. Dabei wurden drei Uferhabitattypen, darunter stark stabilisierte und renaturierte Uferzonen, verglichen und die Nutzung dieser Habitate durch die Fischartengemeinschaft bewertet. Die Ergebnisse zeigten, dass auch eine relativ unkomplizierte und kostengünstige

Renaturierungsmaßnahme wie der reine Rückbau von Uferbefestigung erfolgreich sein kann, da dadurch Habitatvielfalt geschaffen wird und wichtiger Lebensraum für die Juvenilstadien von Flussfischarten, einschließlich *Chondrostoma nasus*, entsteht. Diese Studie lieferte außerdem wichtige Erkenntnisse für zukünftige Renaturierungen von Uferzonen in Flüssen mit stark schwankendem Abfluss, was insbesondere im Hinblick auf die prognostizierten Auswirkungen des Klimawandels von großer Bedeutung ist.

Die Ergebnisse dieser Dissertation belegen, dass Schutzstrategien für Süßwasserfische ganzheitlich ausgerichtet sein sollten und alle Ebenen der Biodiversität, den kompletten Lebenszyklus der Zielart und die Multidimensionalität von Fließgewässern integrieren müssen. Dazu zählt einerseits, eine genaue Kenntnis der Ökologie der Zielart zu haben, denn diese ist – wie die Dissertationsergebnisse zeigen – auch bei relativ gut erforschen Arten wie *Chondrostoma nasus* immer noch lückenhaft. Andererseits ist es von entscheidender Bedeutung, gewählte Schutzmaßnahmen umfangreich zu evaluieren. Hierbei gilt es, sich ihrer jeweiligen Stärken und Schwächen bewusst zu sein, während es ebenso wichtig ist, ihren Erfolg zu bewerten, indem man das gesamte Instrumentarium der verfügbaren konventionellen und modernen Möglichkeiten des Monitorings nutzt. Neben den neu gewonnenen Erkenntnissen zur Ökologie von *Chondrostoma nasus* lassen sich aus den Ergebnissen der Dissertation zusätzlich konkrete Empfehlungen ableiten, um das zukünftige Schutzmanagement für den Erhalt der Art umfassend zu verbessern. Dazu gehören die Wahrung genetischer Integrität im Rahmen von Nachzuchtprogrammen, wie auch die Renaturierung von Schlüsselhabitaten und die Verbesserung der Konnektivität der Teillebensräume in vertikaler, longitudinaler und lateraler Dimension. Diese Ergebnisse sind in weiten Teilen auch auf andere rheophile Flussfischarten übertragbar. Da alle im Rahmen dieser Dissertation untersuchten Maßnahmen in ihrem Wirkpotenzial räumlich und zeitlich begrenzt sind, sollte sich das generelle Paradigma im Erhaltungsmanagement von Süßwasser-Biodiversität von der Fokussierung auf Zielarten lösen und sich zukünftig in Richtung eines prozessorientierteren Erhaltungsmanagements entwickeln. Eine solche Verschiebung, würde es ermöglichen, eine deutlich umfassendere und nachhaltigere Skalierung von Schutzmaßnahmen zu gewährleisten.

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1. General Introduction

1.1 Current status of freshwater ecosystems and biodiversity

Water is the most widespread resource on earth, yet only 2.5% of the world's water volume is freshwater, from which the majority is stored in ice, snow, glaciers and permafrost (68.9%) or groundwater basins (29.9%) (Shiklomanov, 1991). Only 0.26% of the world's freshwater resources are available in lakes, reservoirs and rivers, comprising less than 1% of the global land cover (Gleick, 1998). Despite covering only a very small fraction of the planet`s water resources, freshwater ecosystems constitute much of its biodiversity, e.g. ~10% of all known species and one third of all vertebrate species inhabit freshwater ecosystems (Dudgeon et al., 2006; Strayer & Dudgeon, 2010), which makes them particular prone to species loss (Dudgeon, 2010; Figure 1). In addition, freshwater ecosystems provide fundamental goods and services that support human civilization (Aylward et al., 2005; Geist, 2011), including clean water for drinking and food production, supply of harvestable food sources, transportation opportunities, nutrient and flood regulation, carbon sequestration, dilution of pollutants, and recreational services (Postel & Carpenter, 1997). Freshwater ecosystems are therefore essential for human survival and not substitutable (Carpenter et al., 2011). They are, however, intensively used and altered (Carpenter et al., 2011), which has resulted in them being listed among the most threatened ecosystems on earth (Allan & Flecker, 1993; Dudgeon et al., 2006; Vörösmarty et al., 2010).

The intensive anthropogenic use of freshwater ecosystems results in a variety of stressors that particularly affect stream ecosystems (Figure 2). They include hydrologic flow modification (e.g. by damming, hydropower use, channelisation), land-use change, chemical pollution, water abstraction, introduction of invasive species, exploitation of fish and invertebrates as well as climate change effects (Bierschenk et al., 2019; Carpenter, 2011; Dudgeon, 2010; Heino et al., 2009; Mueller et al., 2020). These stressors pose an acute threat to freshwater ecosystems and their biota, being well reflected in the dramatic and deepening decline of aquatic biodiversity, which is by far exceeding that of terrestrial ecosystems (Collen et al., 2014; Dudgeon et al., 2006). The decline of freshwater biodiversity is evident on a global scale (Tickner et al., 2020), but particular in the densely populated and industrialized areas of

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Europe and the USA (Deinet et al., 2020; Harrison et al., 2018). Freshwater fish extinctions in the USA and Europe are more than a hundred times higher than natural extinction rates, as shown in a study by Dias et al. (2017). The freshwater biodiversity crisis affects all trophic levels, from primary producers to macroinvertebrates and fishes (e.g. Jähning et al., 2021; Körner, 2002; Moyle & Leidy, 1992; Su et al., 2021), although species losses from higher trophic levels such as fishes occur more frequently than from lower trophic ones (Petchey et al., 2004).
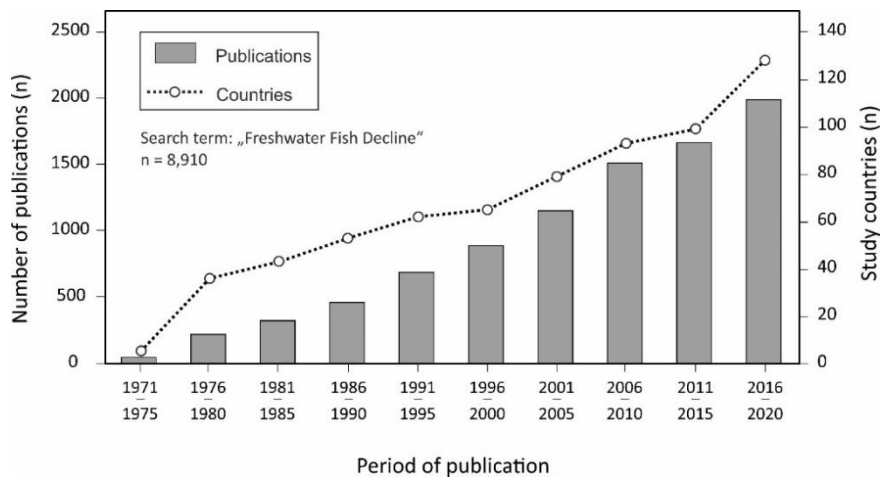
Figure 1: Number of publications (bars) and study countries (dots) related to the search term "Freshwater Fish Decline" based on the results of literature research in the Web of Science database (conducted at 20 December 2021)

Fishes comprise more than 32,000 documented species, from which almost 50% (~15,000 species) are considered freshwater ones (Nelson et al., 2016). A recent report of the World Wildlife Fund for Nature (WWF) and other non-governmental organisations suggests that recent counts of freshwater fish are even higher (more than 18,000 species) and exceed those of saltwater species (WWF, 2021). From all freshwater fish species that have been assessed for their conservation status by the International Union for Conservation of Nature (IUCN) until the end of the year 2021 (n = 11,211; accessed 30 December 2021), around 25% are currently threatened with extinction or even have become extinct (IUCN, 2021)[1]. The steep decline of freshwater biodiversity has prompted policies, preliminary in industrialised countries, which enshrine the protection of freshwater ecosystems in law since decades[2]. In addition, a strategic plan for global biodiversity conservation was agreed in 2010 under the

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[1] The term "threatened with extinction" comprises the categories "critically endangered" (n = 629), "endangered" (n = 932), and "vulnerable" (n = 1,042).
[2] E.g. the European Habitats Directive (Council of the European Communities, 1992), the European Water Framework Directive (Council of the European Communities, 2000), the US Endangered Species Act (16 U.S. code, 1973), the US Clean Water Act (33 U.S. code, 1972).

Convention on Biological Diversity, a platform established and supported by the world's governments (McCarthy et al., 2012). This plan included 20 conservation targets to be achieved over a 10-year period by 2020, including aims to manage fish stocks and their habitats in a sustainable way. Until now, these efforts have largely failed to halt the global trend of freshwater biodiversity loss (Reid et al., 2019). As a consequence, scientists from around the world recently issued urgent warnings about the still ongoing decline of aquatic biodiversity and propose both, immediate action from state and non-state actors (Harrison et al., 2018; Tickner et al., 2020) and advances in global freshwater biodiversity research to improve conservation actions (Maasri et al., 2022). Maasri et al. (2022) advocate for a priorisation of freshwater research on 15 pressing needs, including the identification and tackling of gaps in biodiversity knowledge and the investigaton of the response of biodiversity to multiple stressors.
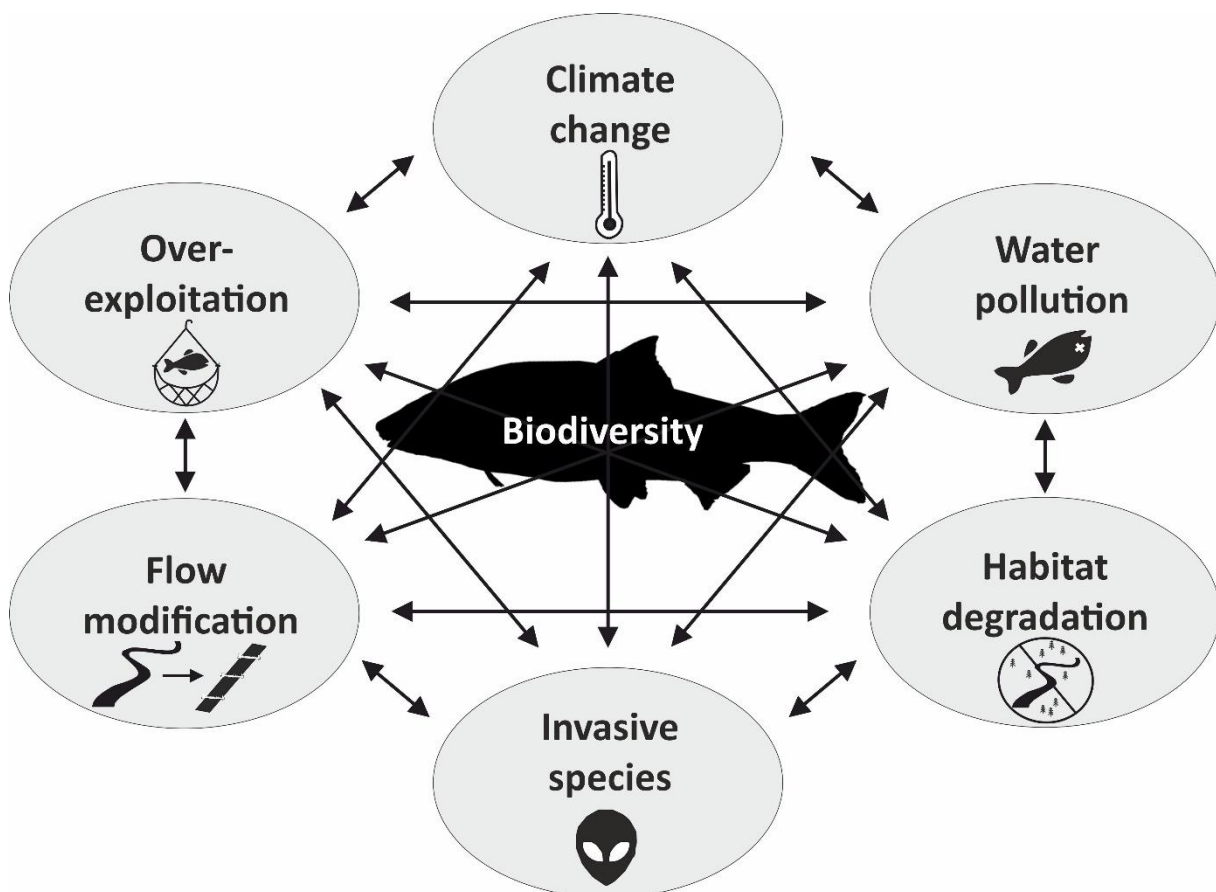
Figure 2: The major threats affecting freshwater fish biodiversity and their established or potential interactions (modified from Dudgeon, 2006)

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Although the chemical, biological and hydromorphological restoration of rivers has faced increasing attention in recent years (Arthington et al., 2010; Geist & Hawkins, 2016; Ormerod, 2004), river modification especially in the context of hydropower use is high on the agenda in some of the world's largest rivers, that are considered hotspots for biodiversity (Geist, 2021; Vander Sleen & Albert, 2022). This holds particularly true for the Amazon (2,406 named fish species; Jézéquel et al., 2020), the Congo (1,200 named fish species; Harrison et al., 2016) or the Mekong (924 named fish species; Valbo-Jørgensen et al., 2009). The predicted loss of fish distribution, diversity and biomass associated with the planned hydropower installations in these rivers (Barbarossa et al., 2020) will also affect protein availability in the riparian states and is estimated to increase the demand for water abstraction, agricultural land and greenhouse gas emissions for compensating farming (Begossi et al., 2018; Orr et al., 2012). This clearly illustrates that complex interdependence of factors related to the management of freshwater resources.

Several studies have reported that stressors to aquatic ecosystems do not only act on an antagonistic level but also cause synergistic effects, making it increasingly difficult to detangle cause and effect relationships (Birk et al., 2020; Côté et al., 2016; Jackson et al., 2016), which in turn marks a mandatory basis to tailor effective conservation strategies for freshwater biodiversity. At the same time, uncertainty about what freshwater biodiversity actually is and what levels and components it comprises often further hampers the success of conservation efforts (Christie et al., 2006; Gaston & Spicer, 2004; Geist, 2011).

1.2 Novel concepts of freshwater biodiversity organisation

Although the public understanding of biodiversity has evolved in the last decade, it is still characterised by emotional media reports about the decline of iconic species such as polar bears (Roe, 2019). Consequently, there is still a lot of misconception about what biodiversity really means. In the public perception the term biodiversity is widely understood as the number of species in a given habitat unit (Geist, 2011), which is also commonly agreed among conservation biologist as a useful starting point for defining biodiversity (Christie et al., 2006). This definition of biodiversity can be easily assessed by evaluating "species richness", which is

the most simple and straightforward measure of biodiversity (Krebs, 2009; Geist, 2011). However, taxonomic uncertainties (Harper & Hawksworth, 1995) and the varying extend of a habitat unit (mikro-, meso-, or makro scale) can still complicate it (Christie et al., 2006; Whittaker, 1977). Species richness is further defined into alpha diversity, referred to as number of species in a given area, beta diversity, as the turnover in species richness when comparing two habitat areas, and gamma diversity, the species richness in an entire region (MacArthur, 1965; Whittaker, 1972).

In recent years, the concept of biodiversity interpretation has been further expanded to account for the actual high diversity and complexity of biodiversity (Christie et al., 2006; Geist, 2011). Novel concepts structure the different aspects of biodiversity into hierarchical levels starting with **genetic diversity** of individuals, populations or entire ecosystems, followed by **intraspecific diversity** expressed as phenotypic or behavioral variation, **community diversity**, **ecosystem diversity**, and finally the **diversity of functional processes**, which is interacting with all other levels of biodiversity (Figure 3).
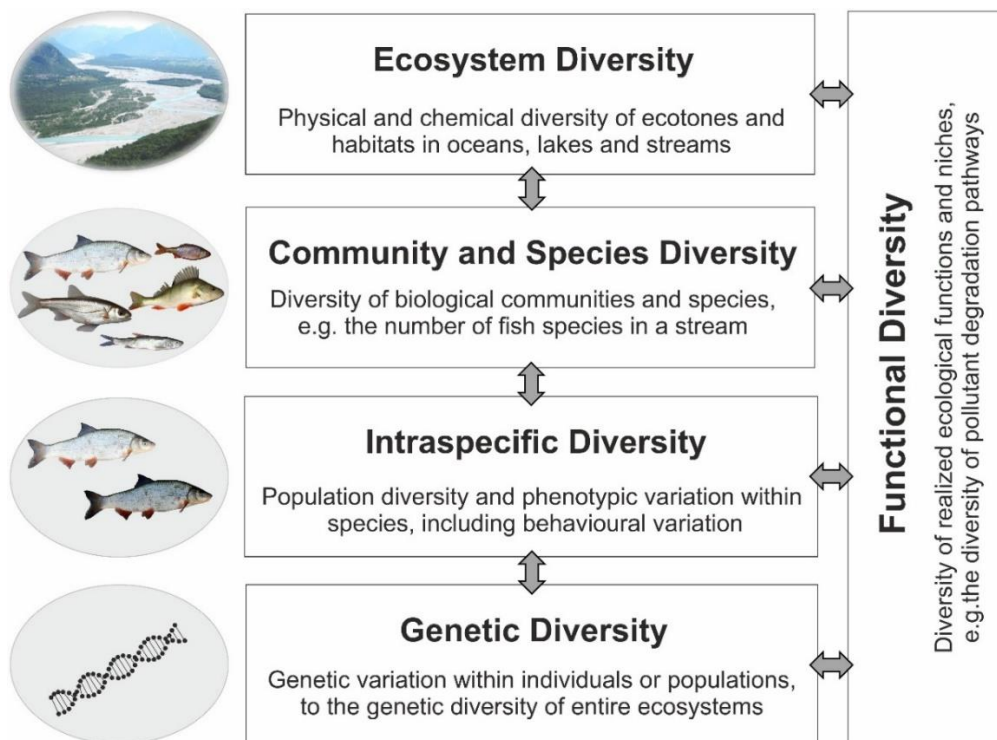
Figure 3: Hierarchical levels of biodiversity organisation in aquatic ecosystems (modified from Geist, 2011)

Genetic diversity determines the number of inherited traits in a species, population or ultimately in an entire ecosystem. A high genetic variation is key for the adaptive potential to

changing environments (Willi et al., 2007). In freshwater ecosystems, this holds particularly true for species inhabiting lentic ecosystems in temperate regions, where streams show a great fluctuation in temperature, discharge and matter fluxes, governing a high seasonal and spatial variability in habitat conditions (Lorenz et al., 1997; Tockner et al., 2003; Schiemer et al., 2020). **Intraspecific diversity** is related to the phenotypic and behavioral variation of populations within a single species. Phenotypic variation can occur between different populations in the same river catchment, as evident from populations of *Chondrostoma nasus* in Switzerland. There, populations show a clear variation in body shape depending on their origin from streams flowing into Lake Constance, the Lake Constance itself and the Lower Rhine downstream of Lake Constance, which was explained with local adaption to different habitat conditions (Hudson et al., 2014). Intraspecific diversity also refers to behavioral patterns as observed in a study by Ovidio and Philippart (2008), in which the majority of radio tagged *Chondrostoma nasus* showed similar restricted movement patterns of just ~300 hundred meters during a five weeks observation period, while single individuals moved several kilometers in the same time. **Community diversity** refers to the number of species in a given habitat, commonly explained as species richness. However, species richness has to be interpreted with caution. An increase in this index does not necessarily mean an increase in biodiversity value, since a high number of species is often associated with a high share of generalist species to the cost of more specialised ones (Erős, 2007). An example for this development is the alpine River Inn, where species richness showed only slight changes in the last decades while abundance changed drastically from highly specialised fish species towards less specialised and ubiquitous ones (Pander et al., 2021). **Ecosystem diversity** explains the chemical and physical diversity of habitats in an ecosystem. Riverine ecosystems are typically characterised by a great variety of habitat conditions, yet have been widely uniformed by anthropogenic alterations (Bunn & Arthington, 2002). As a result, very few existing streams retain the natural functioning of widely pristine rivers. Two of such kind are for example the River Tagliamento in Italy and the River Vjossa in Albania. Both rivers are still characterised by a highly dynamic mosaic of various aquatic and terrestrial habitats (Tockner et al., 2003; Schiemer et al., 2020), matching the diverse ontogenetic habitat requirements of specialised fish species. **Functional diversity** includes manifold processes and phenomena, which, among others, comprise trophic interactions (Duffy et al., 2007), pollution degradation pathways (Geist, 2011), and natural disturbance from fluvial dynamics (Ward et al., 2001). A typical

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example in stream ecology are floods, which lead to a nutrient and water exchange in the floodplain (known as the "flood pulse", Junk et al., 1989), but also to a relocation of stream bed substrate, which is crucial for the functionality of the hyporheic zone, a key habitat in riverine ecosystems (Denic & Geist, 2015; Sternecker et al., 2013ab).

Owing to evolutionary processes some change and loss of biodiversity is normal. The current extinction rates, however, are up to 1000 times higher compared to anthropogenic undisturbed reference rates (De Vos et al., 2015), which highlights the fundamental crisis of biodiversity loss. In stream ecosystems, the alteration of the flow continuum and the associated habitat loss and disruption of functional processes have been identified as a main anthropogenic cause for vanishing aquatic biodiversity (Bunn & Arthington, 2002). Furthermore, the overexploitation of certain species and the introduction of invasive species (Hermoso et al., 2011) put additional pressure on declining populations. In the future, as more and more evidence indicates, climate change will pose the greatest threat to aquatic, but also to terrestrial biodiversity (Arneth et al., 2020). Biodiversity conservation strategies therefore need to embrace all levels of biodiversity and at the same time target all causes of biodiversity loss.

Despite the existence of such holistic and integrative concepts (see Geist, 2011), most conservation approaches are still either based on one aspects of biodiversity (e.g. whether community diversity richness matches reference indices), or focus on single charismatic species and the restoration of specific habitats, e.g. restoring spawning grounds for salmonids (Colléony et al., 2017; Geist, 2011; Pulg et al., 2013). Hence, they are rather static and restricted and run the risk of missing the conservation targets, as defined by the Convention on Biological Diversity in 2010 (McCarthy et al., 2012). Another reason for failing conservation efforts, particular for freshwater fish, is the insufficient knowledge on life stage specific traits of threatened species (Smialek et al., 2019). This holds particular true for specialised species, which are characterised by a high dependence on the functionality and connectivity of different habitats at different life stages as well as by low levels of tolerance to changing environmental conditions. As such, these species comprise a high vulnerability to environmental stressors but also a good indicator function for the chemical and structural integrity of their habitat, which has made them target species for conservation efforts (Geist, 2015).

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1.3 *Chondrostoma nasus* - a model species to assess the success of freshwater fish conservation

The common nase (*Chondrostoma nasus* L.) is a formerly widespread fish species in Central and Eastern European Rivers. Its native distribution area spans mainly over the basins of Black (e.g Danube catchment), southern North (e.g. Elbe catchment) and southern Baltic Sea (Kottelat & Freyhof, 2007). Although its native distribution area declines due to local extinctions (Hudson et al., 2014; Peňáz, 1996), the current one has been extended into the Rhone (France) and Soca (Italy, Slovenia) catchments (Kottelat & Freyhof, 2007). There, *Chondrostoma nasus* is considered invasive and in the Rhone catchment hybridisations with the endemic *Parachondrostoma taxostoma* (L.) have been reported (Šimková et al., 2013). Despite the expansion of its former distribution range, populations of *Chondrostoma nasus* are threatened in wide parts of its native distribution area (Hudson et al., 2014; Mueller et al., 2018; Peňáz, 1996).

Chondrostoma nasus holds a unique status among the feeding strategies of European freshwater fish, as it is considered the only truly herbivorous species (Gerke et al., 2018). By grazing benthic algae from river substrate it also contributes significantly to the prevention of biogenic colmation processes in eutrophic rivers (Hübner et al., 2020). The trophic position of *Chondrostoma nasus,* however, is not only important in the top-down direction, but also bottom-up, as it comprises an important food source for apex predators (Šubjak, 2013). *Chondrostoma nasus* is considered a rheophilc (Zauner & Eberstaller, 1999) and potamodromus species, with reported average home range of 22.4 km and a maximum home range >40 km (Panchan et al., 2022). Migrations of this species preliminary occur during spawning season or in relation to seasonal changes in discharge and temperature (Huber & Kirchhofer, 1998). Prior to spawning, *Chondrostoma nasus* gathers in huge swarms and subsequently performs upstream migration to suitable spawning grounds with shallow gravelly riffles and adjacent pools, often located in tributaries of large rivers (Rakowitz et al., 2008).
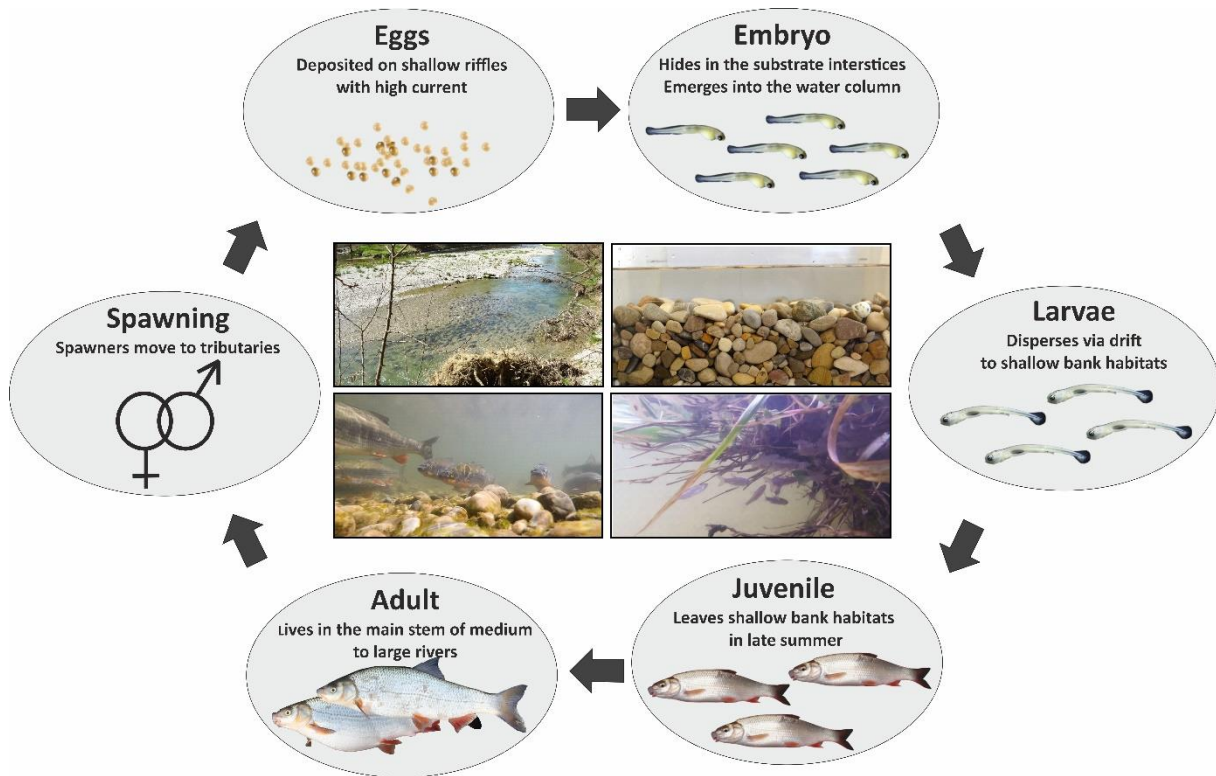
Figure 4: The life cycle of *Chondrostoma nasus* in relation to crucial habitats of the various life stages

There, spawning occurs with huge numbers of spawners per spawning ground (Duerregger et al., 2018; Melcher & Schmutz, 2010) and a sex ratio ranging from 1:3 to 1:20 females to males (Lusk et al., 1995; Peňáz, 1996). Each female spawns up to 20,000 eggs per kg bodyweight (Harsanyi & Aschenbrenner, 1995) by scattering them at the substrate surface where several males immediately fertilise them. Consequently, spawning of *Chondrostoma nasus* is characterised by a high degree of polygyny and polyandry (Peňáz, 1996), which is likely the reason for the observed comparatively diverse genepool (Wetjen et al., 2020). The potamodromus nature of *Chondrostoma nasus* also leads to a high gene-flow and thus a limited genetic variability among populations where no migration barriers exist (Hudson et al., 2014; Wetjen et al., 2020). Owing to its drastic population decline, captive breeding and restocking of *Chondrostoma nasus* has become a widely applied method to support existing relict populations (e.g. Ovidio et al., 2016; Reček et al., 2009).

Compared to other species of the Cyprinidae family, the life cycle of *Chondrostoma nasus* is rather complex, as it relies on a high degree of habitat quality and connectivity in the certain life stages (Figure 4). Consequently, the huge population decline in the past decades is mainly attributed to the anthropogenic alteration of rivers, particular the straightening and damming

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of flow courses, which interrupts migration routes for adults and drifting larvae likewise, disconnects floodplain areas from the main stem and deteriorates spawning grounds (Peňáz, 1996). This development has turned *Chondrostoma nasus* into a target species for freshwater fish conservation and an indicator species for the assessment of river restoration measures, which holds specifically true for the early life stages as these are suspected to pose the major bottleneck for successful reproduction (Schiemer et al., 2002).

1.4 The specific sensitivity of the early life stages of riverine fish species in relation to the four-dimensional nature of lotic ecosystems

In riverine ecosystems, the early life stages of gravel spawning fish species such as many salmonid and rheophilic cyprinid species are particularly vulnerable owing from their specific requirements in the early ontogeny, which rely on a high degree of microhabitat quality, heterogeneity, and connectivity (Schiemer et al., 2002). Thus, these life stages provide a good monitoring tool for microhabitat integrity and the success of river restoration measures (Schiemer et al., 2002). The conceptual framework on the four-dimensional nature of rivers, described by Ward (1989), provides a helpful structure to illustrate the sensitivity of the early life history of riverine fish species. According to Ward (1989), a lotic ecosystem acts in three geometrical dimensions, longitudinal, lateral and vertical as well as on a temporal scale, the fourth dimension (Figure 5). This approach covers matter fluxes and biota interactions and is well suited to be transferred to the spatio-temporal habitat requirements of the early life history of riverine fish.
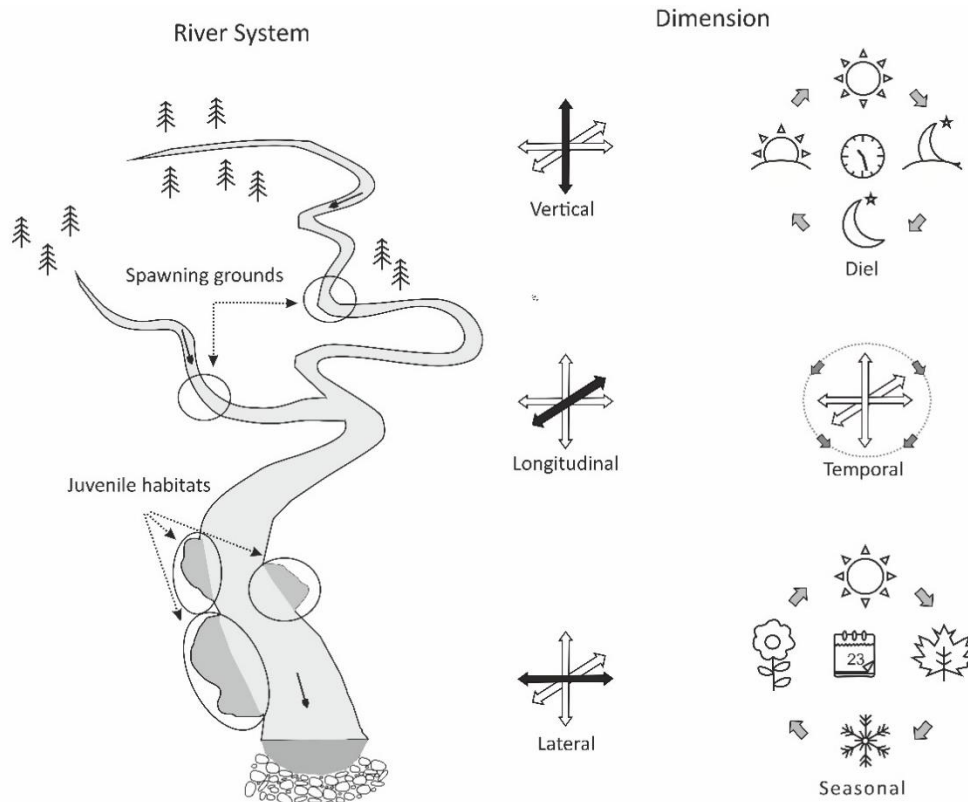
Figure 5: Conceptual framework of the four-dimensional nature of lotic ecosystems according to Ward (1989)

Eggs of these fish species are usually spawned on gravelly riffles with medium to high current, where the eggs are either actively placed (salmonids; Kondolf 2000) or sweep down in the interstitial zone (rheophilic cyprinids; Bless, 1992; Duerregger et al., 2018). At this point, the young fish touches the framework of Ward (1989) for the first time as the egg relies on a loose stream bed structure to conduct this, at this point completely passive, movement into the stream bed in the **vertical dimension**. Connectivity for this life stage is also important in terms of matter fluxes as the eggs require a constant supply of dissolved oxygen during incubation (Greig et al., 2007). After the embryonic development is completed, larvae start hatching and stay in the river bed until emergence. To conduct this, now active, vertical movement from the hyporheic zone into the water column, the young fish still requires a loose and porous bed material (Sternecker & Geist, 2010). Subsequently, larvae enter the current and drift downstream in the **longitudinal dimension**. Fish larval drift is of functional significance for dispersal, gene-flow, and (re-)colonisation of habitats (Lechner et al., 2016; Pavlov, 1994) and is considered as an active-passive transportation process (Glas et al., 2017; Lechner et al., 2014ab; Schludermann et al., 2012). Fish larvae are weak swimmers (Flore & Keckeis, 1998),

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require relatively warm water temperatures (Stoffers et al., 2021) and a specific diet, typically composed of phyto- and zooplankton for further growth (Govoni et al., 1986). To reach functional nursery zones with this characteristic, larvae need to exit the drift in the **lateral dimension** and gather in shallow and low-flow riparian habitats in the floodplain (Balcombe et al., 2007; Nunn et al., 2007; Meulenbroek et al., 2018a; Pander et al., 2017). Finally, riverine fish species are affected by time scales, adding a **temporal dimension** to the conceptual understanding of the early life history of these species. Processes such as the chronology of spawning, the duration of embryonic development and the subsequent larval drift are mainly driven by temperature and annual fluctuations in river discharge, which in turn are subject to seasonal variation, especially in temperate regions (Lorenz et al., 1997; Tockner et al., 2003; Schiemer et al., 2020). The temporal dimension is also evident on a diel scale, as light settings influence the presence of potential predators and thus spawning activity, larval emergence, and drift activity. Time scales also govern important matter fluxes. For instance, re-occurring floods and the related hydro-morphological disturbances are the principal driving force for the quality of the spawning gravel and the existence and productivity of nursery zones (Junk et al., 1989). These examples demonstrate the high vulnerability of the early life stages of riverine fish to the degradation and fragmentation of their habitats, which has manifested itself in sharp population declines of these species, in particular where streams show a high degree of anthropogenic alteration.

2. Objectives

Chondrostoma nasus is a target species for the conservation of freshwater fish biodiversity in European rivers, as the protection and restoration of its populations and habitats is expected to benefit a wide range of other species. This thesis aims at analysing the life stage specific characteristics, habitat needs and possible means of conservation and monitoring to support this species.

Potential conservation tools and monitoring techniques are studied along the hierarchical levels of biodiversity organisation in freshwaters. Moreover, the concept of Ward (1989) on the four-dimensional nature of lotic ecosystems is used to specifically emphasise the importance of habitat connectivity from the egg to the juvenile life stage.

Consequently, the core objectives of this thesis are to:

1. Characterise the various life stages of *Chondrostoma nasus* within and between different populations.
2. Investigate the habitat requirements of *Chondrostoma nasus* in terms of quality and connectivity with respect to the major habitat related stressors in laboratory and field studies.
3. Assessing possible means of conservation for *Chondrostoma nasus* from the genetic to the ecosystem level.
4. Evaluate different monitoring techniques to observe conservation efforts for *Chondrostoma nasus* at various life stages and different levels of biodiversity organisation.

3. General Methodology

3.1   Study concept

A combination of laboratory and field studies was used to study life stage specific characteristics, habitat needs and possible means of conservation and monitoring for *Chondrostoma nasus.* Special emphasis was placed on a combined approach of laboratory studies and the assessment of applied conservation and restoration techniques in natural habitats. First, an ongoing supportive breeding programme of *Chondrostoma nasus* was monitored to assess the genetic integrity of the hatchery-sourced offspring in relation to offspring from the wild (Chapter 4). Second, the size and surface structure of freshly laid eggs of several females from three different spawning populations were examined to gain more information on this important first step of the life cycle (Chapter 5). Third, a laboratory experiment was performed in which green (freshly stripped) eggs of *Chondrostoma nasus* were exposed to various substrate compositions, resembling spawning ground conditions in the wild and substrate fractions commonly used for the restoration of gravelly spawning grounds (Chapter 6). Fourth, a field experiment was conducted to validate the results obtained in the laboratory experiment by testing the effect of spawning ground restoration on recruitment success of *Chondrostoma nasus* in the wild (Chapter 7). Fifth, a comprehensive field study on larval drift in a regulated large alpine river aimed to reveal spatio-temporal and size-specific drift patterns of eggs and larvae from *Chondrostoma nasus* and other riverine species in these river types (Chapter 8). This study also included an assessment of a nature-like fish bypass regarding its intended function as drift corridor and compensatory spawning ground for rheophilic fish species. Last, three bank habitat types in the same river, including restored and stabilised banks, were assessed at different water levels regarding their habitat function for juvenile life stages of riverine fish species, including those of *Chondrostoma nasus* (Chapter 9).

All the work conducted was ethically reviewed and approved by the governmental authorities. Fish sampling was conducted under permit no. 31–7562 (LRA FS to the Aquatic Systems Biology Unit of TUM) following standard sampling procedures to catch and handle fish in

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accordance with national and European guidelines. Fin clipping was done under permit no.
ROB-55.2-2532.Vet_03-20-1.

Table 1*: Overview on studies included in this thesis, regarding the aims, methods used, assessed dimension of river connectivity, assessed level of biodiversity, study type and life stage*

Chapter	Study aims	Methods	Connectivity	Level of Biodiversity	Study type	Life stage
Chapter 4	Assessing the genetic constitution of wild and hatchery sourced offspring	Drift netting of eggs and larvae Genotyping	-	Genetic	Field Laboratory	Larvae Juvenile Adult
Chapter 5	Characterisation of intraspecific egg properties	SEM imaging	-	Intraspecific	Laboratory	Egg
Chapter 6	Evaluation of different substrate compositions on hatching success and larval growth	Flume experiments Measurement of larval size	Vertical Temporal	Ecosystem	Laboratory	Larvae
Chapter 7	Assessment of spawning ground restoration on spawning activity, hatching success and timing of emergence	Freeze cores Drift netting of eggs and larvae Measurement of larval size	Vertical Temporal	Ecosystem	Field Laboratory	Egg Larvae Adults
Chapter 8	Spatio-temporal drift patterns of eggs and larvae in a regulated large alpine river	Drift netting of eggs and larvae DNA Barcoding	Longitudinal Temporal	Community Functional	Field Laboratory	Egg Larvae
Chapter 9	Seasonal bank habitat use in relation to fluctuating water levels	Electrofishing Abiotic habitat characterisation	Lateral Temporal	Community Functional	Field	Juvenile Adults

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3.2   Study area

This thesis includes field and laboratory studies. All laboratory studies were conducted at facilities of the Technical University of Munich, including the Limnological Research Station Iffeldorf (SEM imaging of *Chondrostoma nasus* eggs, Chapter 5), the breeding facility of the Aquatic Systems Biology Unit Weihenstephan (substrate experiment, Chapter 6; stereo microscope based length measurements, Chapter 6 & 7) and the Unit of Molecular Zoology Weihenstephan (genotyping, Chapter 4; DNA barcoding, Chapter 8). Egg and larval material for the studies presented in Chapters 4, 5 & 6 originated from the River Inn catchment in Bavaria (Figure 6). All the fieldwork conducted was performed in the same catchment, comprising sites in the main stem, tributaries and a fish pass. The potential of spawning ground restoration for *Chondrostoma nasus* (Chapter 7) was assessed in two major tributaries of the River Inn, where, according to the current knowledge, the largest spawning runs of this species occur. The field study on spatio-temporal drift patterns of fish larvae in heavily regulated large alpine rivers (Chapter 8) was conducted in the main stem and a nature-like fish pass at the hydropower plant Gars am Inn. Habitat use of altered and restored banks (Chapter 9) was studied in three impoundments spanning over major parts of the flow stretch of the River Inn in Bavaria.
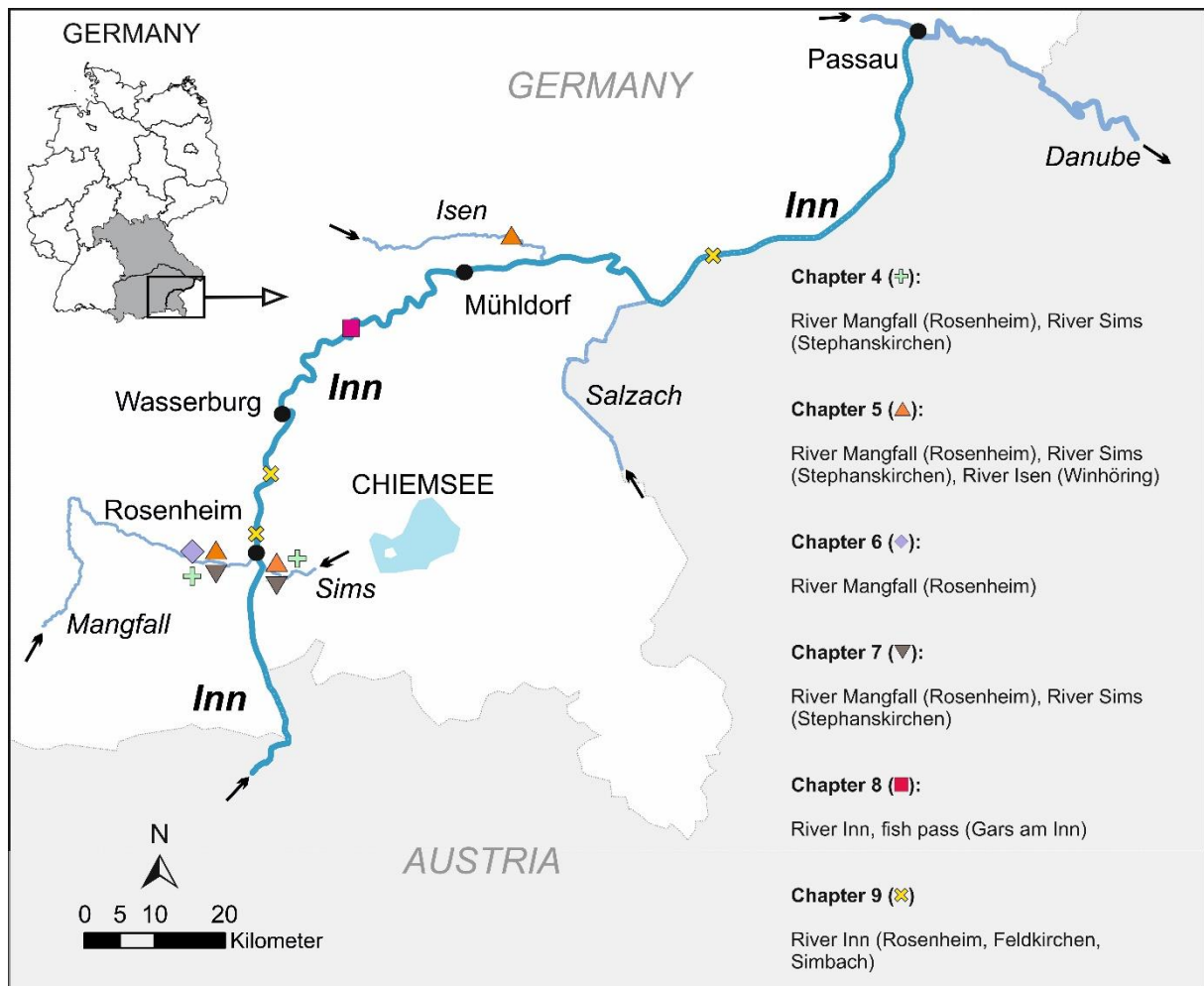
Figure 6: Map of all study sites in the River Inn catchment. Different symbols and colors link the sites to the specific chapters. Note that the symbols of Chapter 4, 5 & 6 indicate sites in which green eggs were obtained for the subsequent laboratory studies. Black arrows indicate direction of flow

The River Inn origins in Switzerland and flows over a river stretch of 517 km through Austria and Germany, where it discharges into the River Danube. It is a snow- and glacier melt dependent alpine river with relatively cold water temperatures (2.0-17.0°C) and a highly fluctuating discharge. Typically, the discharge of the River Inn peaks in summer with high loads of suspended solids, followed by a low discharge and clear water phase in winter. The Inn has been highly altered for the reason of flood protection and hydropower use, which changed the formerly braided river course to a uniform waterbody with a single deep channel. This has led to a loss of the formerly rich habitat mosaic, which encompassed lentic floodplain habitats as well as manifold channels with various depth and flow velocities (Pander et al., 2021). As a result, population declines of specialised fish species in this catchment are mainly attributed to habitat alteration and land use change (Bierschenk et al., 2019; Mueller et al., 2018).

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3.3   Techniques applied in laboratory studies

Eggs and larvae of freshwater fish are usually only a few millimeters in size, making it necessary to use special technologies to study their characteristics. In recent years, technical innovations in the field of high-resolution measures have therefore become of increasing importance to reveal unambiguous species identification in fish eggs and larvae (Ko et al., 2013; Meulenbroek et al., 2018ab), to characterise the structural properties and chemical composition of early life stages (Keckeis et al., 2000), or to trace their feeding ecology (Fuiman, 2021) and movement patterns (Miller, 2021). In this thesis, genotyping of hatchery sourced and wild offspring of *Chondrostoma nasus* was performed to test for the potential genetic effects of anthropogenic selection of adults used for supportive breeding (Chapter 4). Scanning electron microscopy (SEM) was used to study potential intraspecific differences in egg surface structure of *Chondrostoma nasus* eggs originating from different spawning populations (Chapter 5). High-resolution measurements of larval length were conducted under a stereo microscope with the use of specific software (Chapter 6 & 7). Furthermore, DNA barcoding was applied to gain unambiguous species identification of drifting ichthyoplankton (Chapter 8).

3.3.1 SEM imaging

Scanning electron microscopy (SEM) is a commonly applied technique in aquatic science studies when a high resolution of surfaces is needed, e.g. for studying diatom assemblages (Kuefner et al., 2020), gill damage of macroinvertebrates exposed to suspended fine sediments (McKenzie et al., 2020), or intraspecific differences in fish eggs (Riehl & Patzner, 1998).

SEM imaging works with a raster-guided electron beam, which creates interactions between the emitted electrons and the analysed sample. If the analysed sample shows a low conductivity, it must first be coated with a highly conductive material, e.g. gold. The interaction between the emitted electrons and the conductive surface of the sample create high resolution images of the object`s surface, which show a great depth of field in particular (Figure 7). In this thesis, SEM imaging was used to study potential interspecific differences in egg surface properties of *Chondrostoma nasus* eggs by analysing the green eggs of seven females of three different spawning populations (Chapter 5). Sample preparation started with the fixation of the eggs in 96% ethanol right after stripping. Subsequently, they were brought to the laboratory, where egg moisture was removed using a vacuum (0.05 mbar) freeze dryer (Alpha 1–4, Christ) at −47°C for 120 s. Dried eggs were fixed to a SEM sample holder with conductive carbon adhesive pads and gold-coated using a Polaron SC502 Sputter Coater (Fisons Instruments). Subsequently, photographs of the egg surfaces were taken in a standardised grid and under constant technical settings.
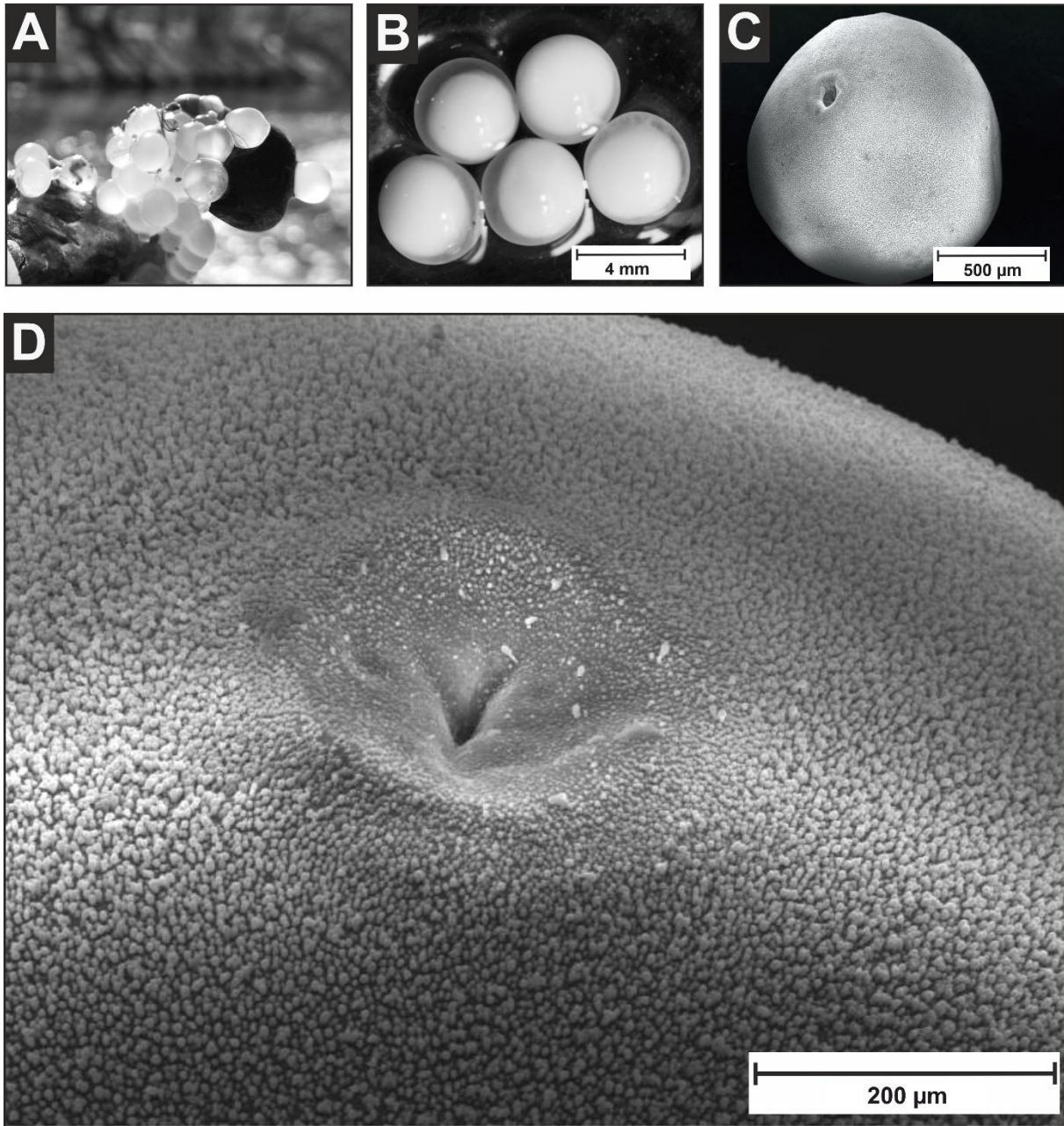
Figure 7: Photographs of *Chondrostoma nasus* eggs (A) freshly laid and collected in the wild from the main spawning ground in the River Mangfall, (B) five hours after spawning photographed with a stereoscope Olympus SZX10 (Olympus Deutschland GmbH) in the laboratory, (C) after fixation (96% ethanol) and vacuum drying, photographed using a SEM, and (D) zoomed to the egg surface with visible attaching plugs and micropyle, again photographed using a SEM

3.3.2 DNA barcoding

Correct species identification in ichthyoplankton based on morphometric criteria only is often subject to great uncertainty, especially in fish eggs, yolk-sack larvae and in areas where eggs and larvae of multiple species co-occur. The use of genetic tools such as DNA barcoding has

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therefore advanced to an important tool in species identification of fish larvae, both in the marine and freshwater environment (Ko et al., 2013; Lira et al., 2022; Meulenbroek et al., 2018ab).

Taxonomic identification of fish larvae in Chapter 8 started with a visual identification to family level. In a next step, larvae of each family were divided into homogenous groups based on the morphometric criteria developmental stage, body shape, pigmentation, shape of head and mouth, and fin position (if developed), by the use of established identification keys (Pinder, 2001; Spindler, 1988). Subsequently, samples of larvae from each identified group and day of occurrence were randomly selected and used for DNA barcoding.

DNA barcoding started with the extraction of DNA from a small piece of tissue (posterior part of the larvae and half eggs respectively), followed by a PCR analysis, where the DNA fragment was amplified. Concentration and purification of the PCR product was assessed via electrophoresis and diluted if required. Thereafter, the DNA fragment was sequenced and the obtained result fed into the NCBI database with a query search and aligned with available sequences. If DNA barcoding confirmed the homogeneity of a morphometrically pre-sorted group (all analysed individuals belong to the same species), the detected species was transmitted to all other individuals of this group for the following data analyses. If DNA barcoding revealed various species in a pre-sorted group, individuals of this group were again checked for morphometric criteria that might explain the different species detected (Figure 8).
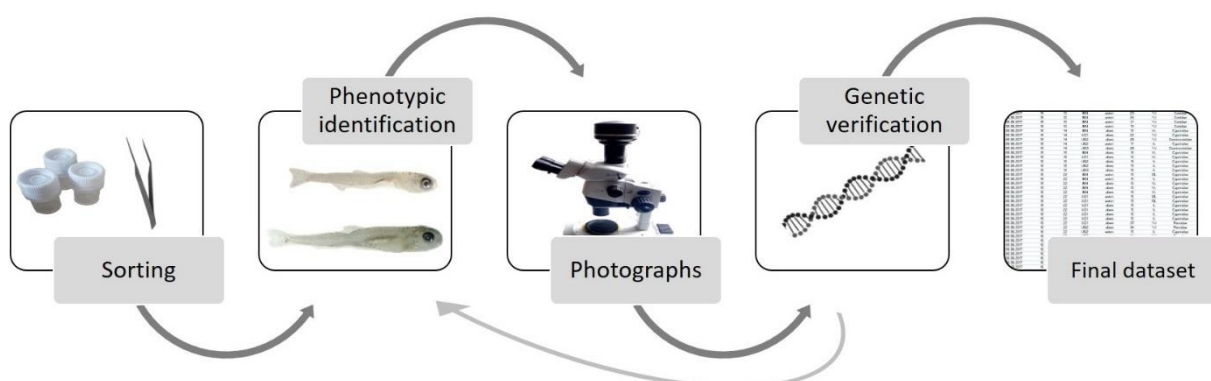
Figure 8: Flow chart explaining the procedure of species identification of fish larvae

3.4   Field studies in natural habitat

Effective restoration of aquatic habitats requires the knowledge of the life stage specific response of the targeted species. Consequently, studies in natural habitats are indispensable when analysing functional relationships of restoration effects and species response.

In this thesis, restoration of *Chondrostoma nasus* spawning grounds in the wild was performed to study the characteristics and possible means of restoration in this very first habitat a young fish encounters (Chapter 7). To account for the next step in the early life history of *Chondrostoma nasus*, spatio-temporal drift patterns of fish larvae were assessed in the heavily modified River Inn and in consideration of a nature- like bypass, aiming to restore longitudinal connectivity and to provide additional spawning habitats (Chapter 8). Finally, bank habitat restorations for enhancing lateral connectivity in the River Inn were evaluated, regarding their function as habitat for juvenile life stages (Chapter 9).

3.4.1 Abiotic habitat variables

To characterise structural and physico-chemical habitat properties in natural habitats of *Chondrostoma nasus* and to assess the abiotic effects of the restoration of its habitats, various abiotic variables were recorded. Measurements included water depth (m), flow velocity 10 cm above ground (m/s), and 10 cm below surface (m/s; recorded with an Ott MF pro, Ott, Kempten, Germany), as well as reading of electric conductance (µS/cm, related to 20 °C), dissolved oxygen concentration (mg/L), pH value (pH), temperature (°C), and turbidity (NTU). These measurements were taken using the handheld devices Multi 3430 (electric conductance, oxygen concentration pH, temperature) and pHotoFlex Turb (turbidity) (both WTW, Weilheim, Germany). Readings of electric conductance, oxygen concentration, pH, and temperature were also used to ensure stable conditions in the experimental flumes in Chapter 6. All measurements in natural habitats were carried out at the same day and shortly after the fish sampling (either drift sampling or electrofishing) was conducted. Each variable was recorded multiple times in each habitat area/sampling stretch/experimental flume, with the numbers of measurements depending on the specific study design.

The study targeting the habitat function of different bank habitat types (Chapter 9) included additional recordings of bed material (block stone, concrete, gravel, sand) as well as the proportion of bank vegetation, dead wood, submerged living roots, and canopy cover. Each of these variables was visually estimated and documented in 5% steps, as described by Pander & Geist (2018).

To test for the effect substrate restoration in Chapter 7, substrate quality was assessed at each studied spawning grounds prior to restoration with freeze-cores (Figure 9). Subsequently, substrate samples were taken at several time points after the restoration using the same technique. All substrate samples were wet-sieved in the fractions of >20–63, >6.3–20, >2.0–6.3, >0.85–2.0 and ≤0.85 mm using an electronic sieving-tower (Fritsch, Idar-Oberstein, Germany). Discrete fractions were dried and weighed to determine percentages by mass. The same technique of substrate fractioning and drying was used in preparation of the composition of standardised substrates in the laboratory experiment in Chapter 5. Freeze cores in Chapter 6 were also used to study the horizontal distribution of eggs and larvae in the hyporheic zone.
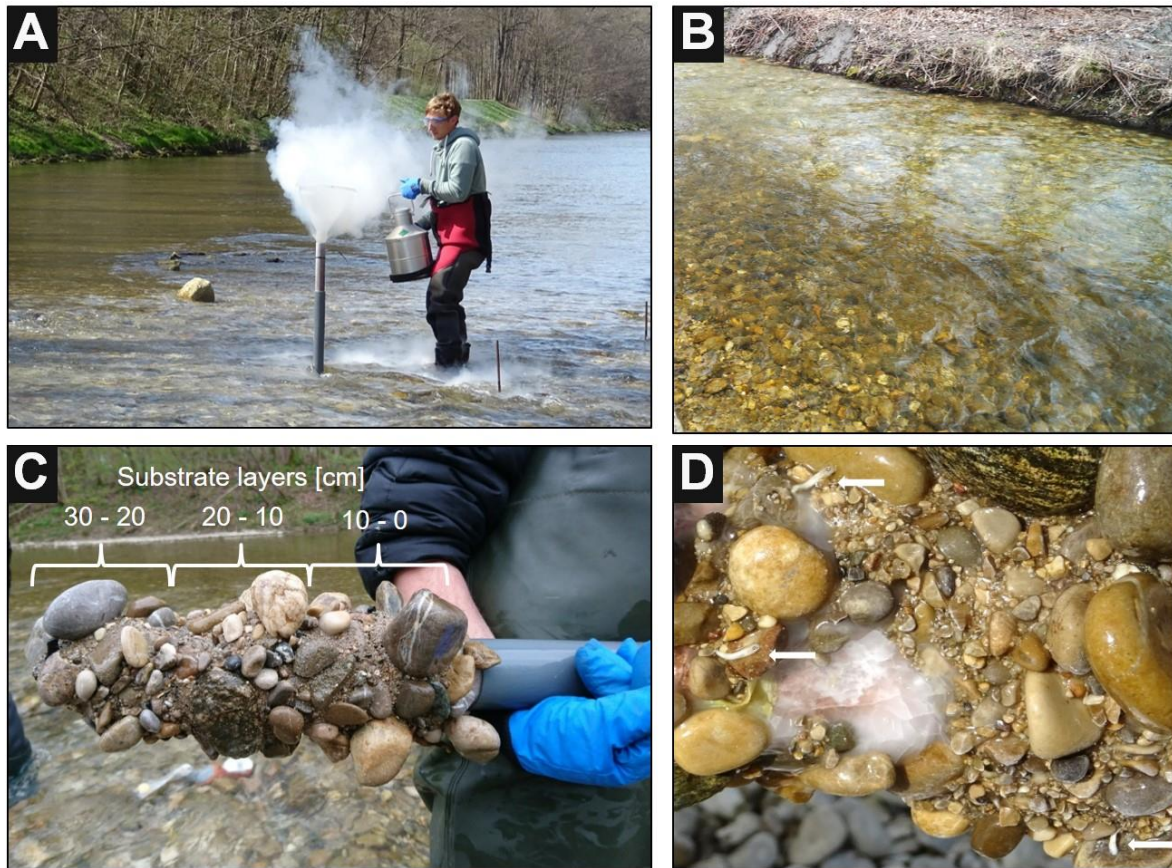
Figure 9: Freeze core sampling using liquid nitrogen (A), visible effects of substrate restoration in the River Sims (B), freeze core from the River Mangfall (C), and freeze core from the River Mangfall including visible fish larvae marked with white arrows (D)

To assess development conditions for eggs and larvae in the hyporheic zone, redox potential (mV) was recorded in studies targeting recruitment potential of different spawning substrates (Chapter 5 & 6). Measurements of redox potential were taken in situ in either 5 cm (Chapter 5) or 10 cm substrate depth (Chapter 6), following the approach described by Geist and Auerswald (2007).

3.4.2 Drift sampling of ichthyoplankton

Drift sampling of ichthyoplankton was done with nets specifically designed for the respective study aims. Drift nets comprised rectangular metal frames as this geometry allows a more precise assignment of drifting ichthyoplankton to lateral or vertical drift paths in the water column, e.g. compared to rectangular net openings (Meulenbroek et al., 2018a). Fine-meshed

(155 meshes cm^{-2}) and tear-resistant polyester was used for the nets, which was stiffened at both ends by the use of a highly rigid fabric (Figure 10). A collection bag was tied to each net with a zipper to allow a quick priming and emptying of the drift traps. Drift nets were exposed to the water column by attaching them to iron rods, which were previously anchored in the river bed. Drift sampling was conducted at various light settings, including samples taken during day, dusk, night and dawn (see Chapter 7 & 8). Each sample was checked for fish eggs and larvae directly after sampling in a field laboratory. Eggs and larvae were euthanised using a twentyfold overdose of MS 222 (Tricaine Methane Sulphonate), as recommended by Adam et al. (2013), and subsequently preserved in 96% ethanol. Taxonomic identification was done according to chapter 3.3.2.
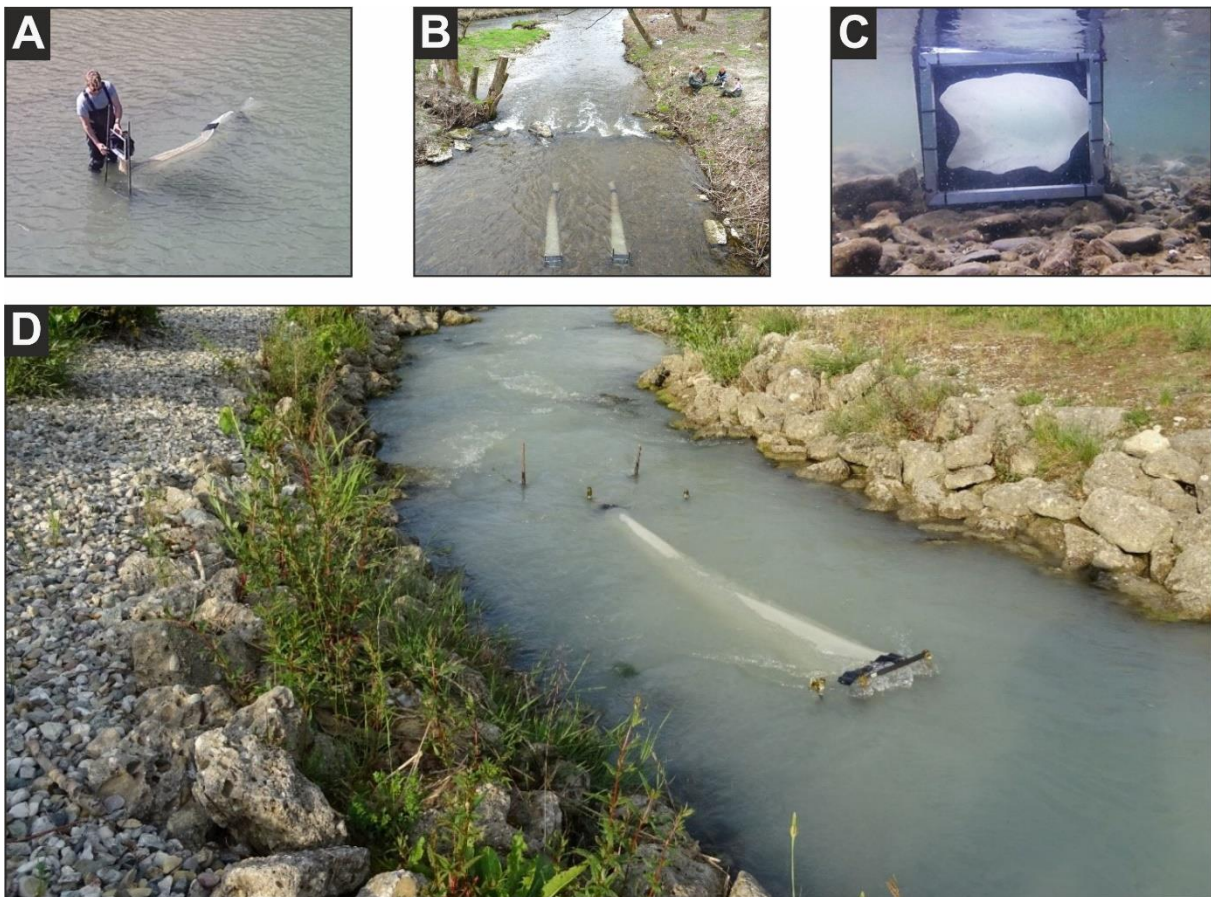
Figure 10: Preparation of drift net sampling by fixing the net on iron rods (A), two drift nets in operation during the spawning ground assessment (Chapter 7) in the River Sims (B), underwater photograph of a drift net opening (C), drift net in operation during the fish bypass assessment (Chapter 8) at the hydropower plant Gars am Inn (D)

3.4.3 Fish sampling of juvenile and adult fish

Sampling of juvenile and adult fish was conducted via electrofishing using either a portable (Chapter 4 & 5; 1.5 kW, Grassl, Schoenau, Germany), land-based (Chapter 7; 8 kW, EFKO FEG 8000, EFKO-Elektrofischfanggeräte GmbH, D-Leutkirch) or boat-based electrofishing generator (Chapter 8; 11 kW, EFKO FEG 8000, EFKO-Elektrofischfanggeräte GmbH, D-Leutkirch). A single hand-held anode was used and stunned fish were collected with a dipnet and transferred to a plastic tank filled with fresh water. Caught fish were determined to species level, measured to the nearest 1 cm and subsequently carefully released at the location from which they had been collected. The comprehensive sampling design of the fish community presented in Chapter 8 & 9 was applied according to Pander & Geist (2010), with replicates of 30 m stretches. Electrofishing was always performed during daylight conditions (8 a.m. – 5 p.m.) and working from downstream to upstream direction. For the genetic monitoring of an ongoing supportive breeding programme, tissue samples (~0.25 cm²) were taken from each adult *Chondrostoma nasus* by clipping the pelvic fin. Scales were used to identify the age of each adult *Chondrostoma nasus* in Chapter 4 & 5.

4. A conservation genetics perspective on supportive breeding: A case study of the common nase (*Chondrostoma nasus*)

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Candidate's contribution:

This study was an equal-authorship publication of Bernhard. C Stoeckle (BS), Melanie Mueller (MM), Christoffer Nagel (CN), and Juergen Geist (JG). BS, MM and CN developed the study design and methodology with constant input from JG. Fin clipping and sampling of fish larvae were conducted by CN. Tables and figures were prepared by CN and BS. The original draft was written and finalised by BS, MM and CN. Revision and editing of the article were done by BS, MM, CN, Ralph Kuehn (RK), and JG.

4.1   Abstract

1. Supportive breeding programmes are becoming increasingly crucial for the conservation of many declining freshwater fishes such as the European common nase, *Chondrostoma nasus*. However, small relict populations are genetically highly vulnerable, and supportive breeding can have a detrimental impact on the genetic composition of the cultured offspring (e.g. as a result of inbreeding, genetic drift, and adaptation to captivity).

2. This study monitored the genetic effects of a continuing supportive breeding programme of common nase by comparing the genetic diversity of two wild spawning populations with

the respective wild offspring and the progeny from captive breeding originating from spawners of the two wild populations, considering genetic variability, genetic differentiation, and inbreeding effects using nine microsatellite markers.

3. Despite low genetic differentiation, the offspring from captive breeding and from one of the natural populations (River Sims) were remarkably different genetically, as indicated by pairwise analyses of genetic divergence (F_{ST} from 0.028 to 0.070; Jost's D_{EST} from 0.080 to 0.205) and the discriminant analysis of principal components. The mean number of alleles and mean allelic richness in the captive-bred offspring and also in the wild offspring of the River Sims were lower than for wild populations of spawners and natural offspring of the River Mangfall, and signs of inbreeding effects were detected ($F = 0.106$ for captive bred and 0.048 for natural offspring).

4. The observed effects can probably be attributed to the limited number of spawners (two females and three males) used for captive breeding. In addition, the results support previous evidence on recruitment problems of the Sims population, such as a reduced hatching success.

5. Collecting fertilised eggs from the wild and rearing them in captivity (repatriation approach) could be an alternative to stripping a limited number of spawners and thereby to improve the conservation of genetic diversity of natural populations.

4.2 Introduction

As a result of the strong decline of many freshwater fish and mussel populations, ex-situ conservation programmes, including captive breeding and restocking, are gaining increasing importance in their conservation (Geist et al., 2021; Lamothe & Drake, 2019; Strayer et al., 2019; Lepič et al., 2019; Manubens et al., 2020; Roques et al., 2018; Targońska et al., 2008; Wetjen et al., 2020). A successful implementation of supportive breeding measures is particularly crucial if populations are declining very quickly or where species are not able to survive in natural habitats despite extensive in-situ habitat restoration efforts (Mameri et al., 2018; Manubens et al., 2020). One example is the common nase, *Chondrostoma nasus* L. (subsequently referred to as nase), a key species of European river systems that was once very

widespread. Nase has a unique role in the food web of central and eastern European rivers, both in the top-down direction by grazing benthic algae, which can significantly increase hyporheic oxygen supply (Hübner et al., 2020), and in the bottom-up direction by providing an important food source for the highly endangered Danube salmon (*Hucho hucho*, L.) (Šubjak, 2013). Nase is considered a medium-age species, which usually reaches an age of 8–15 years (Blahak & Lusk, 1995; Lusk et al., 1995). Sexual maturity is attained in 4–7 years (Lusk et al., 1995) with a fecundity of 15,000 to 20,000 eggs per kilogram body weight (Harsanyi & Aschenbrenner, 1995). Populations of this species began to decrease in the 20th century (Lepič et al., 2019) and have now declined to highly endangered relict populations in many places (Peňáz, 1996; Targońska et al., 2008; Wetjen et al., 2020). This reflects the high sensitivity of rheophilic cyprinids to structural habitat degradation and pollution (Targońska et al., 2008). Restoration measures to improve habitat quality and connectivity (Meulenbroek et al., 2018b; Nagel et al., 2020b; Pander et al., 2017; Ramler & Keckeis, 2019) are currently supplemented by several short-term ex-situ methods to support declining populations and to repopulate rivers after local extinction. These methods comprise the translocation of adults to establish new populations (conservation translocation) (Ovidio et al., 2016; Præbel et al., 2021), and restocking with captive-bred larvae and juveniles (Lepič et al., 2019; Targońska et al., 2008; Wetjen et al., 2020).

Supportive breeding measures in nase and other fish species are usually based on stripping wild spawning adult fish, which are caught by electrofishing, and the release of juvenile progeny later after they have reached a certain size in the hatchery (Targońska et al., 2008; Thorstensen et al., 2019; Wetjen et al., 2020). Spawning adult nase are easily accessible as they congregate to spawn. Nase larvae can be reared in ponds with a high rate of success, which makes supportive breeding and restocking a potentially valuable conservation tool for this species (Mameri et al., 2018; Lepič et al., 2019). However, supportive breeding can have a detrimental impact on the genetic diversity (i.e. loss of genetic diversity) of the offspring generation owing to genetic drift and selection effects (i.e. adaptation to captivity) (Roques et al., 2018; Thorstensen et al., 2019). Selection can occur in different phases of the breeding programme. Capture of potential parents usually takes place only for a limited period (Klupp & Geist, 2018); moreover, only animals that are ready to spawn can be used, and these may not be representative of the population. Fertilisation (artificial mixing of eggs and sperm) and

rearing conditions (e.g. temperature and water chemistry) during incubation may have selective effects in the offspring (Klupp & Geist, 2018). Individuals that are best adapted to the rearing conditions have an advantage, possibly resulting in higher survival rates. Feeding can also be selective, especially when spawners from wild populations are used. In this case, often only a small proportion of juvenile fish accepts the food (Klupp & Geist, 2018). The genetic effects of maladaptation can cause significant physiological and morphological changes (Latorre et al., 2020), eventually resulting in lowered lifetime success compared with individuals hatched in the wild and reduced adaptive potential to changing environmental conditions (Fraser et al., 2019). The use of only a limited number of broodstock individuals not fully representing the genetic diversity of the original population can enhance genetic drift and inbreeding in the offspring (Brown et al., 2000; Franklin, 1980). This has already been demonstrated in captive breeding programmes of other aquatic species using neutral markers (Geist et al., 2021; Rojas et al., 2021). The intentional translocation of populations for conservation purposes can also result in adverse genetic consequences, such as founder effects, genetic drift, and inbreeding depression (IUCN, 2013). It is surprising that only a few studies (primarily focused on salmonids: Rytwinski et al., 2021) have attempted to assess the consequences of this management practice on population genetics (Præbel et al., 2021), yet the controversy about the advantages and disadvantages of supportive breeding and stocking is increasing.

For many freshwater fish species, the effects of artificial reproduction on genetic diversity are well known, and genetically informed management strategies exist (Wetjen et al., 2020), particularly for economically important species such as salmonids. For vanishing species such as nase, which have a limited commercial value or interest to recreational anglers, data are deficient despite their high importance in the food web of European rivers. The genetic structure of nase populations has received limited interest in fisheries management, and data are known only from a few studies in the Rhine catchment in Switzerland (Hudson et al., 2014) and Germany, the Rhône in France (Devaux et al., 2015; Gollmann et al., 1997), and the upper Danube in Germany (Gollmann et al., 1997), all revealing low to moderate levels of genetic differentiation among populations and reduced heterzygosity in several populations (Gennotte et al., 2014).

In this study, a continuing captive breeding programme of nase focused on monitoring genetic effects was assessed. Captive breeding has been carried out by local fishery clubs for several years in two tributaries of the River Inn in Germany (the Mangfall and the Sims), where two of the major remaining spawning sites within the River Inn system are located (Nagel et al., 2020b). Owing to the limited number of ripe females at any given time, the breeding programme typically relies on small numbers of parents, making it necessary to look for genetic effects in the offspring, as observed previously for other species (e.g. Danube salmon; Geist et al., 2009). The nase populations in the Mangfall and Sims have been reported by the local anglers to be morphologically different and to exhibit a distinct homing behaviour. Previous investigations of the effectiveness of restoring spawning areas have shown that spawning sites differ in habitat quality and that these populations differ in demographic structure as well as larval survival rates (Duerregger et al., 2018; Nagel et al., 2020b).

The objectives of genetic monitoring were to (i) assess whether there is genetic differentiation between the two populations, (ii) evaluate the genetic diversity of populations and their suitability for reintroduction, and (iii) test for genetic differences between progeny from captive breeding and wild offspring. These results were intended to be used for suggesting ways of improving future conservation management of the nase populations, and for populations of other rheophilic cyprinids where similar propagation programmes are in place.

4.3   Material and Methods

4.3.1 Tissue sampling and DNA extraction

All the work conducted in this study passed an ethical review and was approved by the government authorities (fish sampling permit no. 31–7562; fin clipping permit: ROB-55.2-2532.Vet_03–20-1).

This study was designed to monitor the impact of the common breeding practice (Harsanyi & Aschenbrenner, 1995) on patterns of genetic variation in nase as described in local rearing guidelines and carried out by the local angling clubs in the River Inn catchment. Spawning nase are caught in close proximity to the spawning sites in two tributaries (Mangfall and Sims) and

gametes are stripped directly in the field. Subsequently, fertilised eggs are brought to the hatchery and reared to a size of ~10 cm before restocking.

Electrofishing was conducted by wading in the River Mangfall (12°6′23.52″E; 47°50′46.66″N; 1 April, 2020) and in the River Sims (12°9′1.02″E; 47°51′4.20″N; 2 April, 2020) using a portable generator (1.5 kW; Grassl, Schoenau, Germany). Prior to further handling, all fish were anaesthetized with MS-222 (tricaine methanesulfonate; concentration according to Adam, et al., 2013). Subsequently, sex was determined and total length (TL) measured to the nearest millimetre. A tissue sample (~0.25 cm2) was taken from each fish by clipping the pelvic fin and immediately preserving it in 96% ethanol. Scales were used to identify the age of each specimen. This procedure was conducted with 30 fish each from the Mangfall and Sims populations (Table 2). In both rivers, the female-to-male ratio was ~1:3, matching the range of sex distribution described in previous studies (Lusk et al., 1995). Mean fish length and age were greater in the Sims population (Table 2).

Table 2: Overview of all treatment groups, including spawning adults from the Mangfall (AM) and the Sims (AS) populations, natural offspring (LM and LS) from these populations, and offspring from the breeding station (BMS). Total length [TL] and age are given as mean values ± standard deviation.

Code	River	Stage	N	Females/Males	TL [mm]	Age
AM	Mangfall	adult	30	12/18	459 ± 31	8.8 ± 1.4
AS	Sims	adult	30	9/21	493 ± 29	9.8 ± 1.6
LM	Mangfall	larval	48	NA	10 ± 1	0+
LS	Sims	larval	48	NA	10 ± 1	0+
BMS	Mangfall/Sims	juvenile	51	NA	102 ± 5	1+

Note: Sex could not be determined in larval and juvenile stages (NA = not available)

In both populations, eggs of two females each were stripped and immediately fertilised by the milt of three males using the 'dry method' (i.e. mixing eggs and milt before adding water after about 1 min). This cross-breeding ratio resembles the artificial reproduction procedure most commonly described in published literature (Keckeis et al., 2000). The total number of females

that can be stripped is restricted by the readiness of the females to spawn, which is limited to a few hours only each spawning season (Harsanyi & Aschenbrenner, 1995). Consequently, several females need to be caught in order to have a few that release eggs. To minimise disturbance of the already overaged and threatened relict population of the nase, the local angling clubs only use the described low number of spawners. As this study was intended to monitor their common practice of artificial breeding, the total number of spawners was not increased for genetic monitoring.

After stripping, egg stickiness was removed by the addition of fresh milk, as described in Targońska et al. (2008). Fertilised eggs were then brought to the hatchery and incubated at a water temperature of 10–12 °C. After hatching, larvae were reared in nature-like ponds until the following spring (see Lepič et al., 2019). To test for the effect of selective breeding on genetic diversity, 51 individuals were sampled from the breeding ponds in spring 2021 with fin clips being used for further genetic analysis (Table 2).

To assess genetic diversity of the natural offspring in the year 2020, two drift nets were placed downstream of each spawning site in the River Mangfall and River Sims 2 weeks after spawning — see Nagel et al. (2020b) for technical details. Drift nets were deployed for 5 days and 2 h each day to catch nase larvae emerging from the spawning sites. Drift nets were also placed upstream of the spawning sites to account for potential bias from larvae drifting from upstream stretches. No larvae were caught in these upstream nets, suggesting that all captured larvae originated from the observed spawning grounds. From all nase larvae caught, 48 were selected from each river for further genetic analysis. To gain representative subsamples of the natural offspring, the larvae selected comprised a subsample from each day of larval emergence, resulting in five subsamples from the River Mangfall and five from the River Sims (Table 2, Supporting information 1). As larval emergence was distinctly higher in the River Mangfall, subsamples represented 10–15% of the total larvae caught each day in this river and ~30% of the total larvae caught each day in the River Sims (Supporting information 1). Genomic DNA was extracted from fin clips and larvae, applying the standard phenol–chloroform method (Sambrook et al., 1989); DNA samples were then stored at −20°C for subsequent analyses.

4.3.2 Polymerase chain reaction and genotyping

Nine microsatellite loci were analysed; these have been used in earlier population genetic studies on freshwater cyprinids (Mesquita et al., 2003; Muenzel et al., 2007; Vyskočilová et al., 2007) and *C. nasus* populations (Hudson et al., 2014). Owing to overlapping allele size ranges of markers, two loci sets were generated for polymerase chain reaction (PCR) multiplexing (Set1: SARN7G5, LSOU08, SARN2F11B, LC290, SARN7K4, LSOU21; Set 2: LSOU05, SARN7F8, LC27) according to Hudson et al. (2014), and forward primers were labelled with three different fluorescent dyes: TAMRA, HEX, and 6FAM.

Multiplex PCR reactions were performed using a Qiagen Multiplex PCR kit (Qiagen, Düren, Germany) in a total volume of 15 µl with the following components: 7.5 µl of the Qiagen-Mix, 3.9 µl (Set 1) and 5.1 µl (Set 2) high-performance liquid chromtography water, 0.2 µl of each primer (0.2 pmol µl^{-1}), and 1.2 µl DNA (20–25 ng µl^{-1}). PCR products were separated on an ABI PRISM 377 Sequencer (Applied Biosystems, Foster City, CA, USA). GeneMapper Software v. 4.0 (Applied Biosystems) was used to score the genotypes.

4.3.3 Data analysis

Microsatellite allele frequencies, the mean number of alleles per locus A, allelic richness AR as a standardised measure of the number of alleles corrected for sample size, expected and observed heterzygosities, HE and HO respectively, and inbreeding coefficient FIS were calculated using Fstat v. 2.9.3 (Goudet, 2001). Genepop v. 4.7.3 (Rousset, 2008) was used to test genotypic distributions for conformance with Hardy–Weinberg expectations using the probability test (Haldane, 1954), and to estimate the significance of genotypic differentiation between these population pairs. All probability tests were based on the Markov chain method (Guo & Thompson, 1992; Raymond & Rousset, 1995) using 10,000 dememorization steps, 100 batches, and 5,000 iterations per batch. The number of distinct multilocus genotypes was determined using the R-package POPPR v. 2.8.348 (Kamvar et al., 2014). Pairwise analyses of genetic divergence (FST and Jost's Dest; Jost, 2008; Wright, 1965) among populations and offspring were made using GENALEX v. 6.5 (Peakall & Smouse, 2012). The R-package Adegenet v. 2.1.1 (Jombart, 2008) in R v. 3.6.2 (R Core Team, 2019) was used to determine mean

individual inbreeding coefficients Find for each source population and the offspring by calculating for each individual the probability of being homozygous at a locus p(h)= F+(1-F) $\sum_i p_i^2$ and summing up log-likelihoods over all microsatellite loci to account for multilocus genotypes, where F refers to the probability that an individual inherited two identical alleles from a single ancestor and pi refers to the frequency of allele i in a population. In addition, relatedness between individuals within populations based on the F-value of the 2mod program (Ciofi et al., 1999) was estimated, quantifying the probability that two alleles share a common ancestor within a population. This measure is correlated with effective population size. The effective population size of the parental generation using the linkage disequilibrium method (Waples & Do, 2010) was estimated with Neestimator v. 2 (Do et al., 2014; Waples & Do, 2008).

The genetic structure of samples was visualised using discriminant analysis of principal components (DAPC; Jombart et al., 2010) as implemented in the software package Adegenet v. 2.1.1 (Jombart, 2008). This method was selected because it is less sensitive to uneven sampling (Puechmaille, 2016). DAPC first transforms the data using principal component analysis and then performs a discriminant analysis on the retained principal components. Results of the DAPC were visualised by the RGB transformation of the three discriminants. Similar generated colours thus correspond to the similar genetic composition of respective individuals or populations (Jombart et al., 2010). The data were assessed for potential genotyping errors, such as null alleles, short allele dominance (large allele dropout), or scoring errors, by using the computer program MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004).

4.4 Results

4.4.1 Genetic integrity and differentiation

The overall fixation index FST was 0.0313 and mean Jost's DEST was 0.0922. Pairwise FST values ranged from FST = 0.0061 (adults from Mangfall [AM]–adults from Sims [AS]) to FST = 0.0700 (natural offspring from Sims [LS]–offspring from breeding station [BMS]). DEST values were generally low, ranging from 0.0180 (AM to AS) to 0.2051 (LS to BMS), with higher levels of differentiation occurring between BMS, LS, and all other populations, as well as natural offspring from Mangfall (LM) (Table 3). All other populations were closely related with maximum FST = 0.0139, as also indicated by the similar colours in the DAPC (Figure 11). The effective population size of the parental generation for LM was 198.8 (parametric 95% confidence interval [CI]: 83.4 to infinite), for LS 19.1 (parametric 95% CI: 14.5 to 25.6), and for BMS 4.3 (parametric 95% CI: 3.5 to 7). The result of discriminant analysis of principal components (20 principal components explaining 87% of the total variation; axis 1: 67%; axis 2: 20%) is consistent with computed FST and DEST values and graphically illustrates the genetic differentiation between source populations from Mangfall (AM), Sims (AS), natural offspring (LM and LS), and offspring from the breeding station (BMS) (Figure 11).

Table 3: Pairwise estimates of Jost's D_{EST}-value (Jost 2008) between populations from rivers Mangfall (AM), Sims (AS), natural offspring (LM and LS) and offspring from breeding station (BMS) above diagonal, with F_{ST} (Weir & Cockerham, 1984) below the diagonal

Code	AM	AS	LM	LS	BMS
AM	-	0.0180	0.0190	0.0800	0.1340
AS	0.0061	-	0.0430	0.1150	0.0811
LM	0.0064*	0.0139*	-	0.0780	0.1492
LS	0.0275***	0.0366***	0.0261***	-	0.2051
BMS	0.0477***	0.0276***	0.0511***	0.0700***	-

*Significance levels: * < 0.05, ** < 0.01, ***< 0.001*
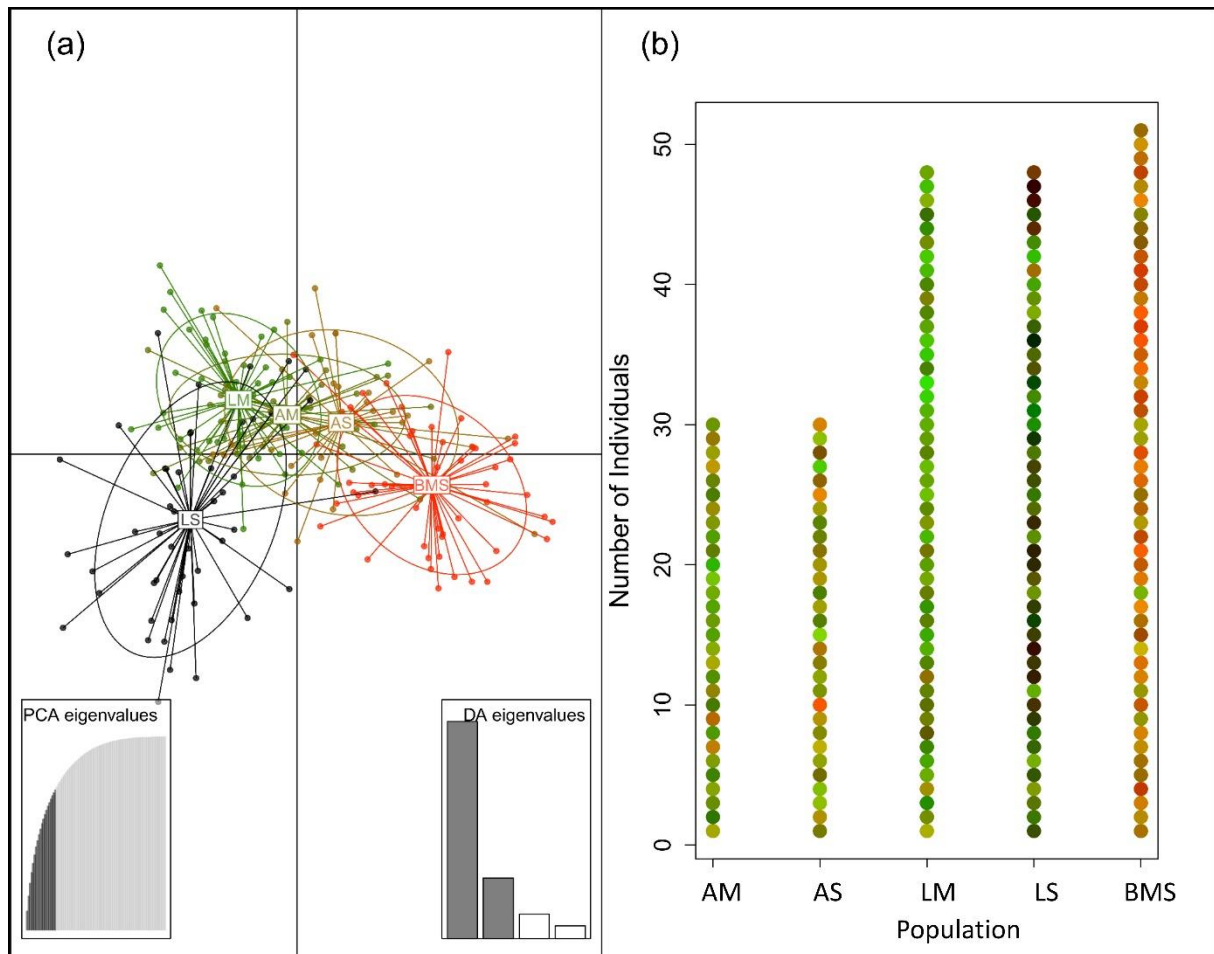
Figure 11: (a) Clustering of Mangfall (AM) and Sims (AS) populations, natural offspring from Mangfall and Sims (LM and LS) and offspring from the breeding station (BMS) based on discriminant analysis of principal components (DAPC) using the first 20 principal components and 2 discriminant functions explaining 87% of the total variation of the data (axis 1: 67%, axis 2: 20%). Individuals are depicted as individual dots; populations are represented by inertia ellipses and mean population colour based on the DAPC. (b) Individual genetic composition of adult individuals from rivers Mangfall (AM), Sims (AS), natural offspring (LM and LS) and offspring from the breeding station (BMS) based on discriminant analysis of principal components (DAPC) using the first 20 principal components and 3 discriminant functions. The colour of the dots corresponds to the results of the DAPC with similar colours indicating similar genetic composition

4.4.2 Genetic variability

MICRO-CHECKER did not detect any genotyping errors or signs of possible null alleles among the dataset. Deviations from Hardy–Weinberg equilibrium were observed both in LS and in offspring from the breeding station (BMS). LS and BMS exhibited a deficit and excess of heterozygotes respectively. Values of observed heterozygosity HO in *C. nasus* populations and their offspring ranged from HO = 0.652 (LS) to HO = 0.769 (BMS). Allelic richness AR ranged from 6.1 (BMS) to 9.0 (AS). The highest individual inbreeding coefficient was detected for LS

(Find = 0.241), and the highest F-values were found in LS (F = 0.048) and BMS (F = 0.106) (Table 4). These latter two samples were the ones that significantly deviated from Hardy–Weinberg equilibrium.

Table 4: Population genetics summary statistics for *C. nasus* populations and their offspring and offspring from breeding station: Number genotyped (N), number of distinct multilocus genotypes (MLG), mean number of alleles (A), mean rarefied allelic richness (A_R), expected heterozygosity (H_E), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}), individual inbreeding coefficient (F_{ind}), F-value based on the 2MOD program (F), and results of Hardy-Weinberg probability tests for deviation from expected Hardy-Weinberg proportions (HW)

Code	N	MLG	A	A_R	H_E	H_O	F_{IS}	F_{IND}	F	HW
AM	30	30	9.1	8.9	0.734	0.741	-0.009	0.185	0.003	
AS	30	30	9.3	9.0	0.762	0.759	0.004	0.181	0.004	
LM	48	48	9.1	8.3	0.740	0.741	0.007	0.190	0.015	
LS	48	47	8.3	7.8	0.740	0.652	0.120	0.241	0.048	***
BMS	51	51	6.7	6.2	0.725	0.769	-0.062	0.175	0.106	***

Significance levels of all tests after sequential Bonferroni correction (Rice, 1989): *** p≤0.001, ** p≤0.01, * p≤0.05

4.4.3 Comparison of captive-bred and natural offspring

Both AR and A were lower in the natural offspring of the Sims (LS) and in the captive-bred juveniles (BMS) relative to their source populations AM and AS (Table 4). Adults and juveniles from Mangfall (AM and LM) and adults from Sims (AS) formed a closely related genetic cluster (Figure 11). High levels of genetic differentiation were observed between LS as well as BMS and all other groups (AM, LM, and AS) (Table 3, Figure 11).

4.5   Discussion

This study shows that first-generation, captive-bred offspring of wild nase exhibit decreased genetic diversity and potential inbreeding effects. This underlines the importance of using genetic tools in conservation and management programmes, especially those that incorporate supportive breeding (i.e. propagation and release). These results indicate that captive breeding should be a management technique of last resort and not the only long-term strategy

for the conservation of threatened fish species. This is also confirmed by the results of a long-term captive breeding programme in the upper Lahn River in Germany, which has been reported to be unsuccessful (Schwevers & Adam, 1997; Wetjen et al., 2020). Most endangered fish species, including nase, exhibit small and declining relict populations, making them especially vulnerable to inbreeding depression and decreased adaptive potential (Fraser et al., 2019; Pavlova et al., 2017; Roques et al., 2018). Under such conditions, genetic monitoring allows the identification of optimal source populations, suitable parental individuals for breeding, and genetic assessment of offspring. In order to ensure the genetic integrity of reintroduced populations, both adult spawners, which represent the gene pool of the original population, and the stocked offspring should be examined (Wetjen et al., 2020).

In the dataset presented here, captive-bred juveniles (BMS) showed a significantly lower mean number of alleles and mean allelic richness than wild populations and a pronounced increase in their inbreeding coefficient (F = 0.106). Moreover, there was evidence of deviations from Hardy–Weinberg equilibrium associated with an excess of heterozygotes, most likely due to the fact that only a few spawners were used (Balloux, 2004). However, no change in heterozygosity could be detected in BMS when compared with wild populations and wild offspring. In nase, reproduction is characterised by polyandric and polygynic spawning behaviour (Peňáz, 1996), theoretically resulting in a high level of genetic exchange in intact river systems. The lower genetic diversity in BMS probably results from the limited number of spawners used in the supportive breeding programme (only two females and three males), resulting in a loss of alleles compared with wild populations. Præbel et al. (2021) showed similar genetic effects for translocated populations of *Coregonus lavaretus* L.

Compared with wild offspring from the River Mangfall, wild offspring from the River Sims had reduced genetic variability, expressed by a lower mean number of alleles, lower observed heterozygosity, increased inbreeding coefficient (F = 0.48), and a deficit of heterozygotes. Results from Neestimator showed that the parental generation of the Sims is smaller (Ne = 19.1) than that of the Mangfall (Ne = 198.8). Previous studies found that the River Sims population exhibited a significantly altered egg surface structure compared with the River Mangfall population (Nagel et al., 2021a), as well as reduced hatching success under controlled incubation conditions (Duerregger et al., 2018), resulting in a significantly reduced recruitment under natural spawning conditions (Nagel et al., 2020b). These outcomes may

have resulted from genetic factors associated with inbreeding or demographic aspects such as the older age of spawners affecting fecundity (Keckeis et al., 2000); however, the levels of genetic variation detected here suggest that genetic factors are important in explaining the difference between these populations. In this study, local fisheries managers mixed offspring from both rivers in the hatchery. This is a commonly used approach because of space constraints, which is well in line with existing guidelines (Harsanyi & Aschenbrenner, 1995). Supportive breeding programmes in nase are only rarely informed by genetic monitoring (Lutz et al., 2021), as these efforts often include private activities of angling clubs, without knowledge transfer between scientists and managers (Lundmark et al., 2019). Using individuals from multiple populations within one river system may increase genetic diversity and the ability to adapt to changing environments, as well as decrease the risk of inbreeding depression, particularly for populations with reduced gene flow (Lutz et al., 2021; *Macquaria australasica*, Cuvier 1830, in Australia; IUCN, 2013). Outcrossing can have positive effects — such as superior fitness of progeny (heterosis) (Whitlock et al., 2000) — if the populations included evolved in similar environments and are not locally adapted (Lehnert et al., 2014), and the degree of genetic divergence among individuals from all the populations is not too high (Præbel et al., 2021). These requirements should be carefully considered before applying an outbreeding approach, as it may otherwise have unintended effects, such as reduced adaptation ability of the offspring (IUCN, 2013). However, differences in local adaptations are highly unlikely for the Sims and Mangfall populations. The two river systems are both sub-catchments of the River Inn, and the spawning sites are only ~5 km apart (Nagel et al., 2020b). It is highly likely, therefore, that the artificial breeding programme in this study area would benefit from including adult breeders from both the Mangfall and Sims populations, while avoiding inclusion of juveniles from the Sims catchment because of their reduced effective population size and associated effects. Cost-effective genetic monitoring is now feasible for evaluating the effectiveness of different management strategies and can inform the decision-making process (Lutz et al., 2021).

There are various ways in which the existing supportive breeding programme can be improved. If the current approach of collecting parents from the wild is maintained, then an increase in the number of parents is critical. This can be realised by increasing the number of spawners in each collection event, especially including a larger number of ripe males, or by

carrying out multiple collection events within the spawning period. A general rule-of-thumb for conservation-oriented breeding programmes is to use 50 male and 50 female spawners (Klupp & Geist, 2018) to minimise the risk of selection and drift effects. These theoretical guidelines conflict with practical recommendations for nase (Harsanyi & Aschenbrenner, 1995) and existing supportive breeding programmes in this species owing to constraints of hatchery space and the availability of broodstock spawners. Consequently, by way of mitigation, rearing activities should be maintained over several years, as this minimizes the risk of genetic bottlenecks, genetic drift, and selection effects, as previously discussed in the captive breeding of endangered freshwater pearl mussel (Geist et al., 2021).

Alternatively, a repatriation approach, where fertilised eggs or hatchlings are collected from the wild and reared in captivity until they have reached sufficient age or size (Thorstensen et al., 2019), may be a more suitable approach for conservation management than using a limited number of adult spawners. This approach maintains natural breeding behaviour, avoids early selection pressures, and reduces domestication selection (Osborne et al., 2020). It also includes more parental lines, increasing the likelihood of representing the genetic diversity found in wild populations, as observed in a study on captive-bred white sturgeon (Thorstensen et al., 2019). Repatriation of early life stages (i.e. eggs or larvae) following a hatchery-based breeding phase has also resulted in lower relatedness among offspring and in a higher number of reproducing adults in lake sturgeon (*Acipenser fulvescens*; Crossman et al., 2011). It was also found to transmit variation successfully from adults through the larvae and into the repatriated population of the razorback sucker (*Xyrauchen texanus*; Dowling et al., 2005). Such management strategies, however, should be applied very carefully, and only with suitable populations. In a first step, validation is needed to test whether the sampling of early life stages of natural offspring truly represents more genetic diversity than captive breeding does.

The effective long-term conservation management of nase and other rheophilic cyprinids should include demographic and genetic monitoring of populations. However, supportive breeding cannot replace in-situ measures to restore natural reproductive capacity (Manubens et al., 2020). It should only be applied, therefore, in addition to instream and catchment restoration measures (Geist & Hawkins, 2016; Knott et al., 2018), as even small-scale habitat restoration, such as cleaning spawning gravel, have proved their effectiveness to increase reproductive success in the wild quickly and effectively (Nagel et al., 2020b). Careful captive

breeding of nase populations may be highly beneficial in streams where populations have declined to a few individuals or have become extinct.

5. SEM images reveal intraspecific differences in egg surface properties of common nase (*Chondrostoma nasus* L.)

Candidate's contribution:

Study design and methodology were developed by CN and Christina Spiessl (CS) with constant input from Joachim Pander (JP) and JG. Sampling and SEM imaging of eggs was done by CN and CS. Data preparation and statistical analysis were conducted by CN. Tables and figures were prepared by CN and CS. The original draft was written and finalised by CN. Revision and editing of the article were done by CN, CS, JP and JG.

5.1   Abstract

Scanning electron microscopy (SEM) has been widely used to describe interspecific differences in egg quality of teleost freshwater fish, but potential intraspecific differences are poorly studied. Eggs of many rheophilic cyprinids are covered with adhesive structures such as attaching villi facilitating egg attachment at substrates of spawning grounds with high currents. Recent findings indicate that the egg quality of the rheophilic cyprinid common nase (*Chondrostoma nasus* L.), a target species of conservation, differs in the adhesiveness between spawning populations, potentially explaining differences in recruitment success. In this study, a SEM image-based standardised protocol was established to assess egg surface quality of *Chondrostoma nasus* eggs. Multivariate statistics detected significant differences of egg surface properties among individual females and among three different populations.

These differences were mainly attributed to length variability and merging of adhesive villi as well as to coating and filament-like connections of these structures. The findings of this study highlight the need for further investigations to better understand the relationship of egg surface properties, egg stickiness and hatching success to understand the recruitment ecology and performance of early life stages in freshwater fish.

5.2   Introduction

Effective conservation of freshwater fish requires knowledge on species-specific traits of each step of the life cycle (Geist, 2011; Pander & Geist, 2013), which holds particularly true for the sensitive egg and larval stages (Schiemer et al., 2002). The life cycle of a fish starts with the release and fertilisation of eggs. The size and structure of fish eggs as well as the timing of release is highly species-specific and evolutionary shaped towards the abiotic and biotic habitat conditions (Bagenal, 1971). While there is a wealth of knowledge on interspecific differences in fish egg properties for a broad set of species of teleost freshwater fish, from temperate to neo-tropical regions (e.g. Brooks et al., 1997; Riehl & Patzner, 1998; Rizzo et al., 2002), very little is known about potential intraspecific variation (but see Keckeis et al., 2000). Also, the egg envelope has been shown to be a sensitive biomarker for environmental pollutants such as xenoestrogens, which may threaten fertilisation and protection of the embryo during development (Arukwe & Goksøyr, 2003; Arukwe et al., 1997). This clearly emphasizes the need for a systematic analysis of the ultrastructure of fish eggs, particularly on an intraspecific level, as water chemical effects on egg development is likely to vary between populations spawning in different rivers.

A widely implemented tool for studying egg surface properties is the use of scanning electron microscopy (SEM) (Riehl & Patzner, 1998; Rizzo et al., 2002). Although the ecology of a species cannot be automatically deduced from structure of the egg surface, eggs of most gravel spawning cyprinid species are characterised by a coverage with adhesive structures such as attaching villi (e.g. Patzner et al., 2006; Petz-Glechner et al., 1998; Riehl et al., 2002). This ensures that a large proportion of the eggs laid adhere to surfaces at spawning sites, which in these species are often characterised by medium to rapid current velocities (Bartoň et al., 2021; Melcher & Schmutz, 2010). This is particularly evident in common nase (*Chondrostoma*

nasus L.) which spawns its eggs at current velocities of up to 1 m/s or even greater (Melcher & Schmutz, 2010; Nagel et al., 2020b). *Chondrostoma nasus* is a specialist among riverine fish and has formerly constituted a large portion of the fish community in many rivers of Central and Eastern Europe (Kottelat & Freyhof, 2007). There, this species plays an important role in the food web for lower trophic levels by grazing on benthic algae (Gerke et al., 2018) as well as for higher trophic levels by providing an important food source for apex predators (Šubjak, 2013). Yet, the degradation and fragmentation of habitats has led to severe population declines of this species (Mueller et al., 2018; Peňáz, 1996). As a result, *Chondrostoma nasus* is listed in several conservation lists (Bohl et al., 2003; Kirchhofer et al., 2007; Wolfram & Mikschi, 2007) and has become a flagship species for river conservation (Schiemer et al., 2002). Spawning of nase occurs in schools in which the sex ratio can reach up to 1 female on 25–30 males (Harsanyi & Aschenbrenner, 1995). During spawning, females scatter large numbers of eggs on the substrate surface of shallow gravel banks where several males immediately fertilize them (Peňáz, 1996). Eggs that are not able to adhere at the surface or the substrate interstices (Duerregger et al., 2018) of the spawning ground drift downstream (Hofer & Kirchhofer, 1996; Nagel et al., 2020b), where development success remains uncertain. Since these eggs are likely to face a higher mortality rate owing from potentially unfavorable development conditions and their availability for drift feeding and weak-swimming predators (Šmejkal et al., 2017), a high proportion of less adhesive and consequently off-drifting eggs may ultimately reduce recruitment potential.

There are anecdotal reports of fish breeders and anglers that suspect great differences in recruitment success of different nase populations. Recent findings also indicate that egg adhesive quality differs between spawning populations of *Chondrostoma nasus* (Nagel et al., 2020b), stressing the need for studying egg surface properties also on an intraspecific level. Consequently, the aim of this study was to investigate potential differences in egg quality of *Chondrostoma nasus* by comparing egg surface properties of seven females originating from three different spawning populations. A standardised protocol sheet for the analysis of SEM images was developed to test our hypothesis that surface structure of *Chondrostoma nasus* eggs shows a significant intraspecific variability.

5.3 Material and Methods

5.3.1 Female spawners

Seven females of *Chondrostoma nasus* were caught in April 2019 during their spawning migration in tributaries of the Inn River (Bavaria, Germany), the largest tributary of the Danube River in Germany (Figure 12). Two females each were caught in the tributaries Isen (48°26'62.74" north, 12°66'16.21" east; April 1st 2019) and Mangfall (12°6'23.52" east; 47°50'46.66" north; April 1st 2019) and three females in the tributary Sims (12°9'1.02" east; 47°51'4.20" north; April 2nd 2019). All fish used for this study were caught in the course of breeding and re-stocking initiatives of local angling clubs using a 1.5 kW electrofishing device (Grassl). Prior to striping of eggs, fish were anesthetised with MS-222 (Tricaine methanesulfonate; concentration according to Adam et al., 2013). Subsequently, total length (TL) of each specimen was measured to the nearest cm and total weight (TW) was determined to the nearest gram. Scales were used to identify the age of each female by counting the annuli. Immediately after egg release, subsamples of ~10 ml unswollen and unfertilised eggs from each female were preserved in 96% ethanol without any contact to water or other substances. Eggs were fixated for at least 10 days prior to further handling.
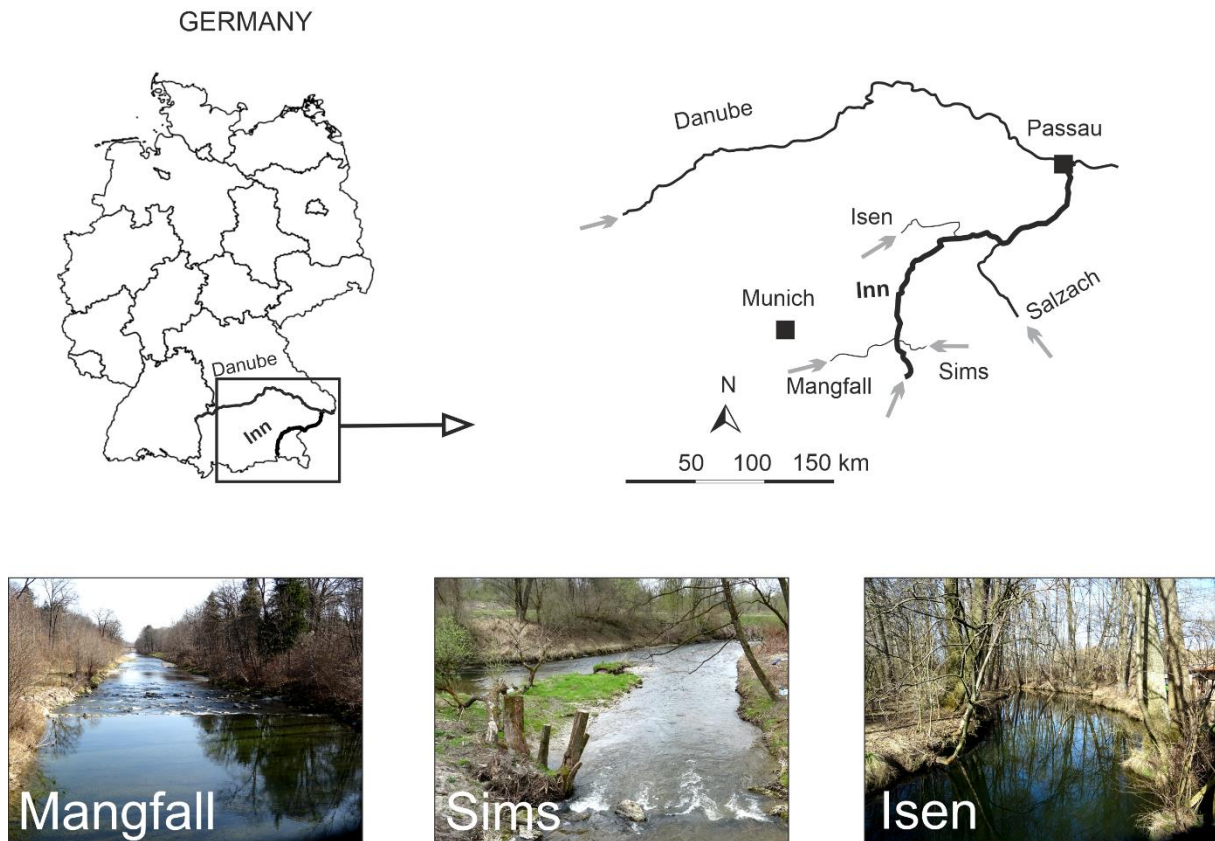
Figure 12: Map of the study area and photographs of the rivers with studied spawning populations of nase

5.3.2 Egg size and SEM imaging

Egg size was determined by measuring the diameter of 15–20 preserved eggs of each female (±0.01 mm) with a stereo-microscope Olympus SZX10 (Olympus Deutschland GmbH) using a magnification of 20.0 and the cellSens-Software (OLYMPUS CORPORATION; www.olympus-lifescience.com). Eggs that were used for these measurement were not taken for subsequent scanning electron microscope (termed SEM hereinafter) imaging to avoid potential bias on egg surface analysis owing from mechanical damage caused by handling of the eggs.

Nine eggs from each female were randomly selected for SEM imaging. First, egg moisture was removed using a vacuum (0.05 mbar) freeze dryer (Alpha 1–4, Christ,) at −47°C for 120 s. Second, eggs were fixed to a SEM sample holder with conductive carbon adhesive pads and gold-coated using a Polaron SC502 Sputter Coater (Fisons Instruments).

Subsequently, eggs were examined with a SEM (S-2300, Hitachi) at a voltage of 25 kV, a geometric working distance of 10 and a magnification of 1,500. Nine photographs of the egg

surface from each egg were taken, following the pattern displayed in Figure 13. Technical settings of the SEM remained constant during imaging of all photographs.

Since the image quality of some photographs was not sufficient for a reliable assessment, which was especially true in the S3 sample, these were excluded. In order to obtain an equal number of images for each egg, seven images from each egg were randomly selected from the remaining photographs. This resulted in a total number of 428 images for the assessment (Table 5). In a final step, all images were encoded and put into a randomised order by an external person. Subsequently, these images were reviewed by the same person and then recoded to their original ID.

Table 5: Origin, ID, female attributes and egg size (mean ± SD) for each specific *Chondrostoma nasus* female used in this study as well as number eggs used for SEM imaging and number SEM images used for egg surface assessment

		Female attributes				**SEM imaging**	
River	**ID**	**TL [cm]**	**TW [g]**	**Age**	**Egg size [mm]**	**Eggs used**	**Images used**
Isen	I1	49	1,384	9+	1.95 ± 0.13	9	63
Isen	I2	47	966	9+	1.81 ± 0.08	9	63
Mangfall	M1	49	1,090	9+	2.22 ± 0.04	9	63
Mangfall	M2	49	1,335	9+	2.11 ± 0.07	9	63
Sims	S1	53	1,660	10+	2.02 ± 0.08	9	63
Sims	S2	54	1,850	11+	2.09 ± 0.07	9	63
Sims	S3	51	1,420	11+	2.21 ± 0.06	8	50
Σ						**62**	**428**

Note: All measurement of egg sizes was done with preserved eggs, which causes a volume reduction of ~ 25% (Patzner et al., 2006). TL = total length; TW = total weight

5.3.3 Assessment of egg surface properties

First, density of adhesive villi (AV) per image was determined by counting the number of AV on the egg surface. For each egg, only the image from the centered photograph was evaluated (Figure 13), as only this shooting angle allowed an accurate counting of all AV. Only fully visible AV were counted.

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⊢ 600 µm ⊣   ⊢ 20 µm ⊣
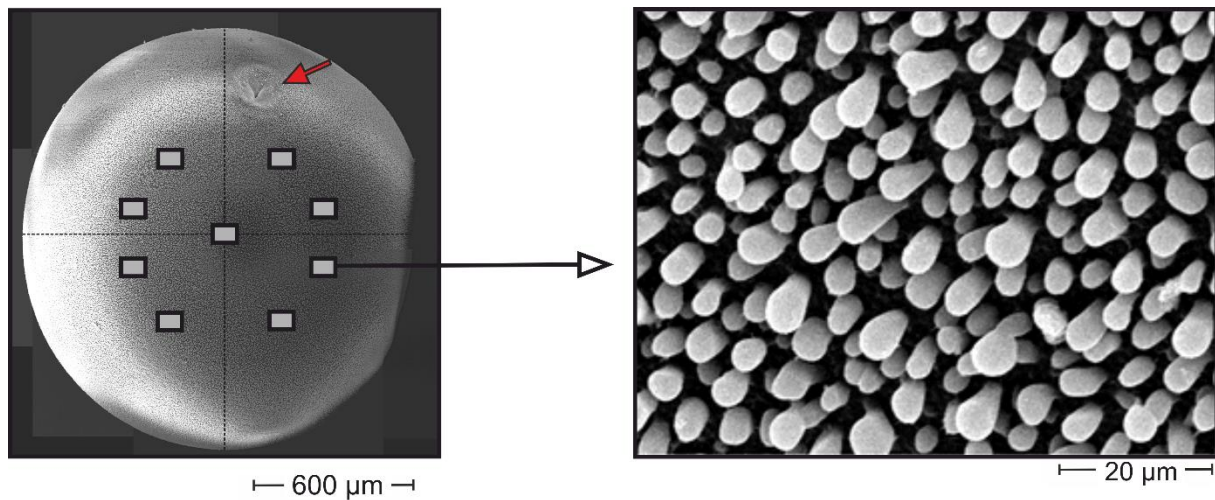
Figure 13: Left side: Egg of *Chondrostoma nasus* (×20) with visible microphyle (red arrow) and an overlaid schematic indicating the areas where photographs were taken. Right side: Magnification (×1,500) used to assess egg surface properties; note the adhesive villi covering the zona radiate externa of *Chondrostoma nasus* eggs

To systematically assess further egg surface properties, six criteria were defined and rated at a level of 0 (low), 1 (medium) or 3 (high). This rating scheme was adapted from a protocol that has been established to assess external injuries in fish and is capable of distinguishing possible differences between groups as well as to identify the underlying causes when combined with multivariate statistics (Mueller et al., 2017a). Assessment criteria were defined according to a combination of results from a literature search (Patzner et al., 2006; Riehl & Patzner, 1998; Rizzo et al., 2002) and own observations on egg surface characteristics. The criteria were: (1) equality of distribution of adhesive villi, (2) length variability of adhesive villi, (3) coating of adhesive villi, (4) merging of adhesive villi, (5) filament-like connections between adhesive villi and (6) globule structures covering adhesive villi (Figure 14, Table 6).

Table 6: Description of the egg surface assessment criteria

Criterion	Description
Distribution of AV	Equality in the distribution of adhesive villi on the zona radiate
Length variability of AV	Estimated variability in the length distribution of adhesive villi
Coating of AV	Adhesive villi are coated with a jelly-like structure
Merging of AV	Merging of several adhesive villi on the distal ends
Filament connections	Filament-like connection between adhesive villi
Globule structures	Small globule structures coat adhesive villi

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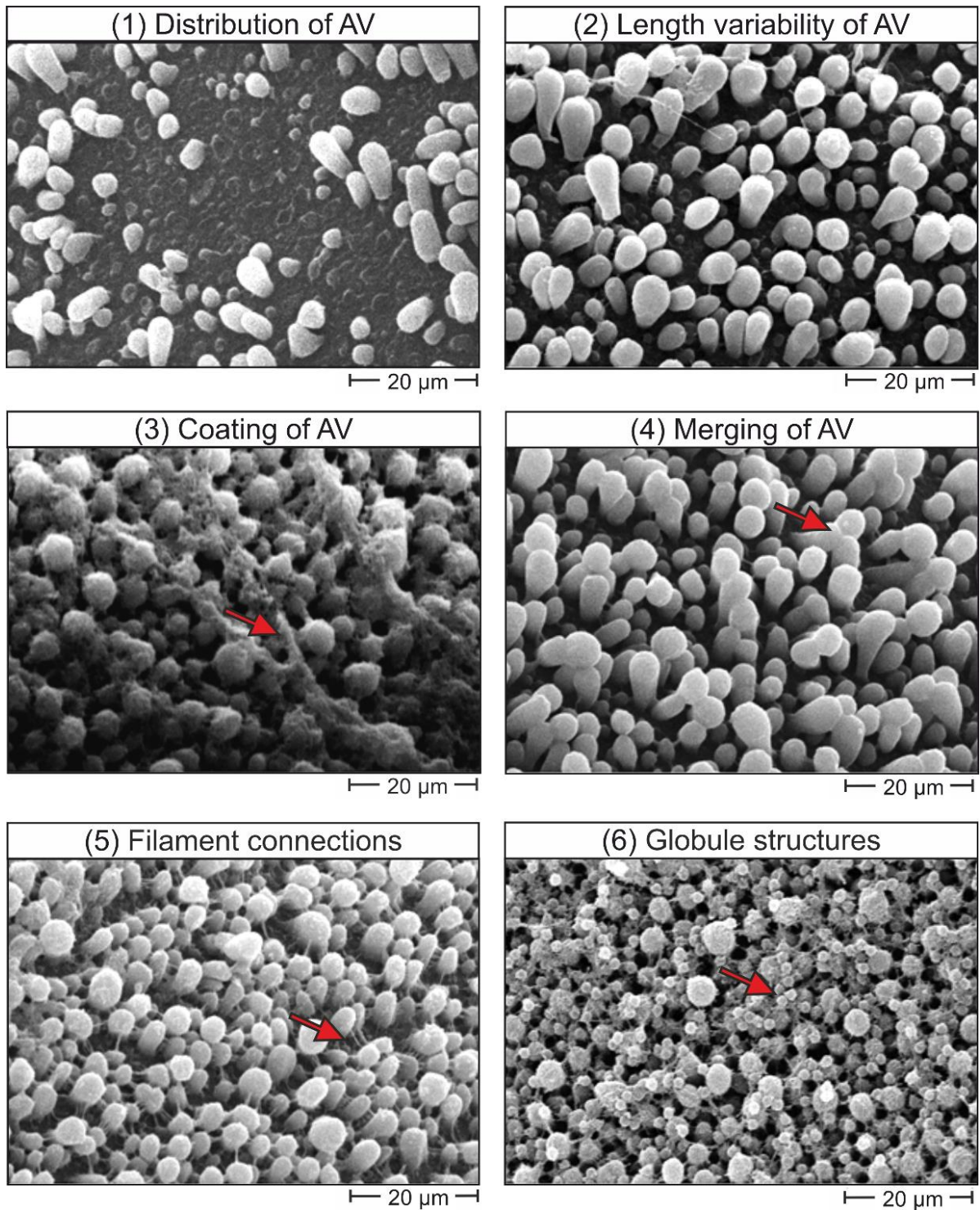
Figure 14: Criteria defined for assessment of *Chondrostoma nasus* egg surface properties. All images represent category 3 (= high occurrence). Red arrows highlight characteristics of criteria 3–6. Definition of the criteria follows Table 6. AV = adhesive villi

5.3.4 Statistical analysis

Univariate statistics were used to test for differences in the densities of AV between individual females and spawning populations likewise. Prior to tests for significance, data distribution was checked for normality using the Shapiro-Wilk test. Since none of the data were normally distributed, significances were tested with the Kruskal-Wallis test, followed by pairwise comparisons using the Mann-Whitney U test. All univariate statistics were performed in R (version 3.6.3; R Core Team, 2017).

Multivariate statistics were used to compare egg surface properties according to the criteria of the assessment protocol described above. First, a resemblance matrix based on Bray-Curtis similarities (Bray & Curtis, 1957) was computed using each image as a sample and each assessment criterion as a variable. Non-metric multidimensional scaling (nMDS) was performed to visualise differences in egg surface properties. The one-way analysis of variances (ANOSIM) was used to check for significances in egg surface differences of individual females and spawning populations. Subsequently, a similarity percentages analysis (SIMPER) was performed to reveal the criteria causing similarities and differences in and between the groups. All multivariate analysis were conducted in Primer v7 (Plymouth Marine Laboratory). For all analysis, significant differences were accepted at $p < .05$.

5.4   Results

From a total of 59 SEM-images analysed, density of AV varied from 150 to 379 per image, which equals 31,250 to 78,832 AV per mm². Significant differences were detected on the level of individual females (Kruskal-Wallis-Test: χ2 = 29.058; df = 6; p < 0.001) and populations (Kruskal-Wallis-Test: χ2 = 7.650; df = 2; p < 0.05). When comparing individual females, AV density was lowest in M1 (171 ± 10) and significantly higher in all other *Chondrostoma nasus* (Figure 15).
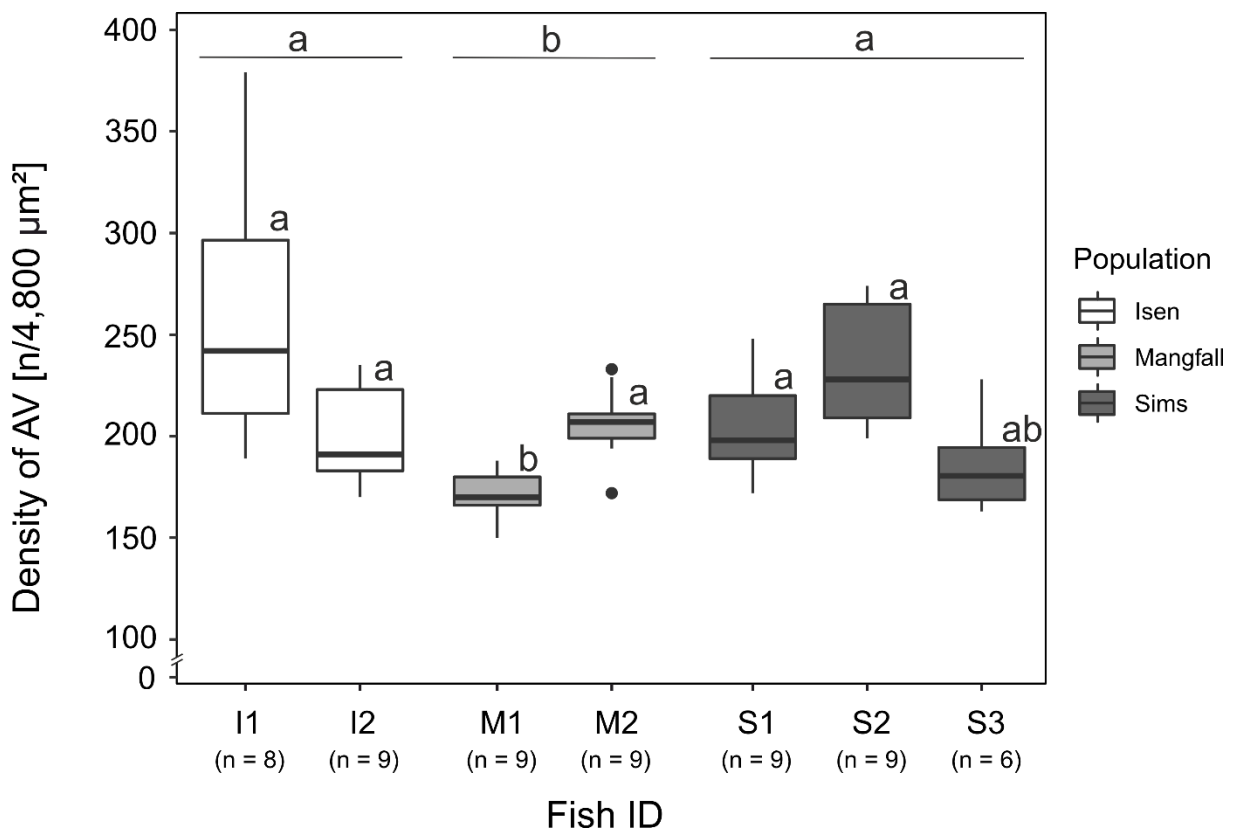
Figure 15: Density of adhesive villi (AV) on the zona radiate externa. Spawning populations are indicated by different shades of grey. Outliers are marked with black dots. Unequal small letters above boxes and indicate statistically significant differences between different females and spawning populations respectively (p ≤ .05). Abbreviation of the IDs follow Table 5

Based on a total of 428 images assessed according to the criteria of the protocol, ANOSIM detected significant differences between eggs from different populations (Global R: 0.32; p < 0.001) and overall females likewise (Global R: 0.34; p < 0.001; Table 7, Figure 16). Differences were most pronounced among eggs from the Mangfall population with the Sims and Isen populations, but only small differences occurred in the comparison of females originating from

the Isen compared to the River Sims, as reflected by the widely overlapping ordination of the symbols in Figure 16 and the low R value of this group comparision of only 0.064 (Table 7). Egg surface images from the Isen population revealed an average similarity of 49.8%, to which filament-like connections between AV contributed most (contribution: 57.02%; average rating: 1.62), followed by length variability of AV (contribution: 17.36%; average rating: 0.66). Eggs from the Sims population showed an average similarity of 51.33%; mainly caused by a high prevalence of filament-like connections between AV (contribution: 56.73%; average rating: 2.16) and coating of AV (contribution: 14.82%; average rating: 0.96). Average similarity in the Mangfall population was highest (54.65%) and, contrasting to the Sims and Isen populations, mainly caused by length variability of AV (contribution: 49.53%; average rating: 1.80) and merging of AV (contribution: 19.72%; average rating: 0.87). Consequently, these criteria also caused the differences in the comparisons between the populations and individual females (Table 7).
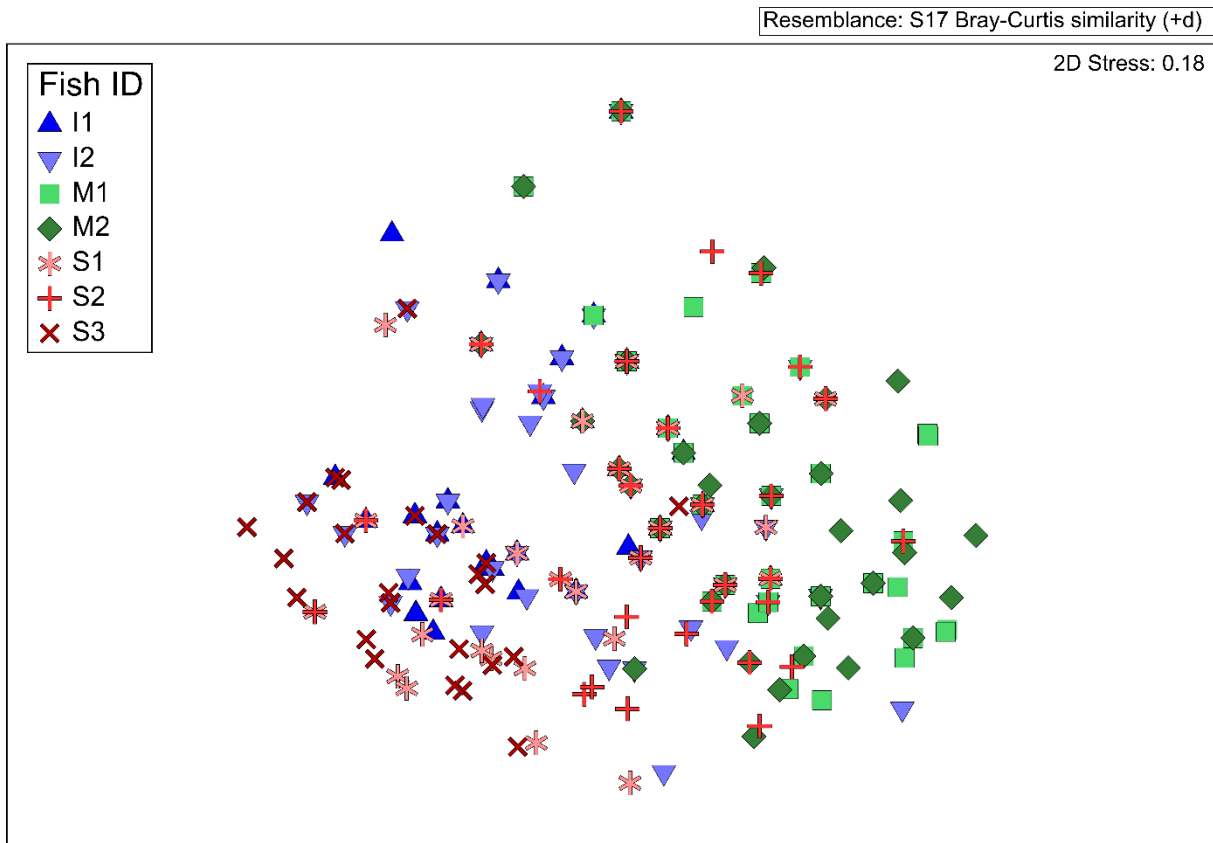
Figure 16: Non-metric-multidimensional-scaling (nMDS) of egg surface properties for different females (indicated by different symbols) and spawning populations (indicated by different colors). The distance between the symbols corresponds to the dissimilarity in egg surface properties (small distance = large similarity). Abbreviation of the IDs follow Table 5

5. SEM images reveal intraspecific differences in egg surface properties of common nase (Chondrostoma nasus L.)

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Table 7_:_ Group comparisons of the different populations and individual females. R- and p-value are based on the one way analysis of similarities (ANOSIM). Average dissimilarity (AVDIS) and ranked criteria contribution (given in %) is based on the results of the similarity percentages (SIMPER) analysis

Comparison	ANOSIM		AVDIS	Ranked criteria contribution [%]		
	R	p		1st	2nd	3rd
Population						
Isen vs. Mangfall	0.316	**<0.001**	60.37	Length variability [26.6]	Filament connections [25.3]	Distribution of AV [16.2]
Isen vs. Sims	0.064	**<0.001**	51.82	Filament connections [27.7]	Coating [18.6]	Length variability [16.8]
Mangfall vs. Sims	0.353	**<0.001**	62.47	Filament connections [29.2]	Length variability [21.6]	Coating [14.8]
Individual females						
I1 vs. I2	0.088	**<0.001**	51.57	Filament connections [30.9]	Length variability [17.7]	Distribution of AV [14.6]
I1 vs. M1	0.445	**<0.001**	62.24	Filament connections [31.3]	Length variability [27.7]	Merging of AV [14.8]
I1 vs. M2	0.390	**<0.001**	58.70	Filament connections [30.2]	Length variability [20.2]	Distribution of AV [18.5]
I1 vs. S1	0.158	**<0.001**	47.63	Coating [31.0]	Filament connections [26.0]	Length variability [15.5]
I1 vs. S2	0.181	**<0.001**	51.81	Filament connections [29.1]	Length variability [21.1]	Merging of AV [17.9]
I1 vs. S3	0.315	**<0.001**	46.62	Globule structures [33.1]	Filament connections [27.0]	Length variability [20.6]
I2 vs. M1	0.330	**<0.001**	63.09	Length variability [32.7]	Filament connections [19.9]	Merging of AV [16.0]
I2 vs. M2	0.223	**<0.001**	57.43	Length variability [32.7]	Distribution of AV [19.7]	Filament connections [19.6]
I2 vs. S1	0.192	**<0.001**	53.67	Filament connections [26.9]	Coating [26.6]	Length variability [16.0]
I2 vs. S2	0.074	**<0.01**	53.95	Length variability [24.0]	Filament connections [19.9]	Merging of AV [18.2]
I2 vs. S3	0.432	**<0.001**	57.77	Filament connections [33.4]	Globule structures [26.0]	Length variability [11.4]

5. SEM images reveal intraspecific differences in egg surface properties of common nase (Chondrostoma nasus L.)

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M1 vs. M2	0.061	**<0.01**	46.48	Length variability [30.7]	Distribution of AV [23.3]	Merging of AV [18.6]
M1 vs. S1	0.493	**<0.001**	63.78	Filament connections [28.7]	Coating [24.5]	Length variability [23.5]
M1 vs. S2	0.153	**<0.001**	53.03	Length variability [29.4]	Filament connections [23.4]	Merging of AV [18.8]
M1 vs. S3	0.855	**<0.001**	76.81	Filament connections [33.6]	Globule structures [22.1]	Length variability [20.1]
M2 vs. S1	0.390	**<0.001**	57.72	Filament connections [29.3]	Coating [23.0]	Length variability [19.5]
M2 vs. S2	0.087	**<0.001**	48.61	Length variability [26.2]	Filament connections [23.5]	Distribution of AV [20.4]
M2 vs. S3	0.839	**<0.001**	74.14	Filament connections [32.6]	Globule structures [22.3]	Length variability [15.7]
S1 vs. S2	0.161	**<0.001**	49.99	Filament connections [27.0]	Coating [25.0]	Length variability [20.6]
S1 vs. S3	0.492	**<0.001**	51.33	Globule structures [29.0]	Filament connections [24.9]	Coating [17.0]
S2 vs. S3	0.630	**<0.001**	62.18	Filament connections [27.1]	Globule structures [26.0]	Length variability [15.6]

5.5   Discussion

The findings of this study point at distinct differences in the surface structure of *Chondrostoma nasus* eggs among populations and individuals, which likely affect adhesiveness and thus recruitment success in this species. The reasons for these differences may be explained by genetic effects such as local adaptation, by maternal effects or ambient environmental conditions which needs to be clarified in future studies. The protocol developed in this study has demonstrated its applicability to assess egg surface properties and, when used in combination with multivariate evaluation methods, its ability to identify potential intraspecific differences in the egg surface structure of *Chondrostoma nasus.* Egg quality in general is affected by several components, ranging from endocrine status and diet composition of the female during growth of the oocyte, nutrient composition of the oocyte to female attributes such as size and age as well as physico-chemical water conditions affecting egg incubation after egg release (Brooks et al., 1997; Keckeis et al., 2000). Yet, an effect of the latter can be excluded in our study, as eggs were directly striped and fertilised without any contact to water. However, a variety of reasons remain that could explain the differences observed. Keckeis et al. (2000) found that egg size and to a lesser extend also the chemical composition of the egg is highly influenced by the age of the female spawner. As female *Chondrostoma nasus* of the Sims population were older (10–11 years) than females from the Mangfall and Isen population (all 9 years), this could also explain the differences observed in our study, which were mainly caused by higher occurrence of filament-like connections, coating of the egg surface as well as a lesser length variability of AV in the Sims population. Yet, the rather small differences in age of 1–2 years suggests that this is unlikely to be the case and stresses the need for further investigations. Future research should also include endpoints such as stickiness and hatching success, as it remains unclear if these are related to the differences in the observed eggs surface properties. However, previously observed differences in adhesive abilities (Nagel, et al., 2020b) and hatching success between the Mangfall and the Sims population in the wild (Duerregger et al., 2018) suggest that this is likely the case. This stresses the need of linking observations on egg surface properties to general egg quality expressed by egg stickiness and hatching success and other important incubation conditions such as physico-chemistry of the water (Kincheloe et al., 1979; von Westernhagen, 1988) and substrate composition (e.g. Nagel

et al., 2020a; Sternecker & Geist, 2010). This is of particular importance as severe recruitment problems may arise from a combination of stressors such as a poor egg quality, a reduced adhesive ability resulting in higher off-drift of eggs and deteriorated habitat conditions on spawning grounds. Additionally, recent findings demonstrate that egg adhesiveness at spawning grounds can be extremely reduced in rivers with hydropeaking effects (Bartoň et al., 2021). In turn, improvement of spawning ground quality might partially compensate for reduced egg quality as a loose and porous interstitial as well as low fine sediment infiltration rates positively contribute to hatching success (Nagel et al., 2020ab). In addition, a porous spawning substrate can incorporate a higher share of laid eggs, even if they have less adhesive abilities, and eggs infiltrating to the hyporheic zone are incubated in more sheltered conditions compared to those, that could not adhere at spawning sites (Duerregger et al., 2018; Persat & Olivier, 1995).

In light of still declining *Chondrostoma nasus* populations and intensive efforts to conserve and restore this species, future research is needed to better understand the relationship between egg surface properties and constraints for recruitment success in the early life history of this species. Assessing differences in egg surface properties in relation to adhesiveness and recruitment success in species with similar eggs other than nase may also be an important future direction in understanding fitness differences and resilience among individuals, populations and species in relation to changes of their habitats.

6. Substrate composition determines emergence success and development of European nase larvae (*Chondrostoma nasus* L.)

Candidate's contribution:

Study design and methodology was developed by CN with constant input from JP, MM and JG. CN conducted the experiment. Data preparation and statistical analysis, including all figures and tables, were conducted by CN. The original draft was written and finalised by CN. Revision and editing of the article were done by CN, JP, MM and JG.

6.1   Abstract

European nase (*Chondrostoma nasus*) is a specialist riverine fish, characterised by a complex life cycle making it vulnerable to habitat degradation. Recent findings indicate that, analogously to salmonids, the interstitial zone quality may pose a serious bottleneck for successful recruitment of this species. In this study, nase eggs were exposed to different substrate qualities. First, standardised substrate mixtures with differing fine sediment additions were used. Second, we tested different homogenous gravel fractions for their influence on egg development and emergence success. In both setups, substrate composition significantly affected emergence success, timing of emergence and larvae size at emergence. In the substrate mixtures, emergence was most successful in substratum with no fine sediment addition (98%) and decreased to 55% in substratum with 20% fine sediment addition. Emergence was most successful in the coarsest fraction (93%) and decreased to 47%

in the finest fraction. Over all treatments, the time between hatching and emergence from substrate differed by up to 156 degree days, thereby indicating that free embryos of nase use the shelter of the interstitial zone for early ontogeny. These results suggest that a loose and porous stream bed can positively contribute to the development success of eggs and larvae and thereby potentially improve the recruitment of nase populations. It is thus important to consider the substrate and interstitial conditions in the conservation and restoration management of this rheophilic cyprinid.

6.2   Introduction

Degradation of habitats has been identified as one of the main reasons for the decline of riverine fish species (Bunn & Arthington, 2002), particularly in highly industrial European countries (Aarts et al., 2004). The completion of a fish's life cycle is reliant on the availability, quality and connectivity of habitats for the different life stages. This is especially true for all riverine species with a complex life cycle, such as the European nase (*Chondrostoma nasus*, L.*;* subsequently referred to as nase), a flagship species for the conservation of European rivers (Schiemer et al., 2002). Beside the accessibility of functional spawning grounds and nurseries (Melcher & Schmutz, 2010), the quality of such habitats is crucial for the completion of the most critical life stages of riverine fish species in general and nase in particular (Keckeis et al., 1997; Pander et al., 2017; Peňáz, 1996). The interstitial zone on spawning grounds is well known to be a key habitat for the early life stages of salmonid fish species in river ecosystems (Geist, 2011; Kemp et al., 2011; Pander & Geist, 2013), as well as for other biota, such as freshwater mussels (Geist & Auerswald, 2007). In many rivers in Central Europe, the porous space of the interstitial zone is clogged and colmated. Main reasons include land-use change and increased erosion (Wood & Armitage, 1997) as well as modifications of in-stream flows and processes (Auerswald & Geist, 2018) often linked to river damming and channelisation (Wharton et al., 2017). This ultimately results in a decline of habitat quality of the interstitial zone due to physical blocking effects by colmation and compaction (Sternecker & Geist, 2010), bio-geochemical effects of reduced oxygen supply (Denic & Geist, 2015; Sear et al., 2017) as well as water temperature effects that interact with the recruitment success of gravel spawning salmonids (Sternecker et al., 2014). To date, most of the studies analysing the effects

of fine sediment on fish have focused on the salmonid family (e.g. Kondolf, 2000; Phillips et al., 1975; Soulsby et al., 2001; Sternecker et al., 2013ab), but the knowledge is rather scarce for gravel spawning cyprinids. Lithophilic gravel spawners from the Cyprinidae family, such as nase or barbel (*Barbus barbus*, L.) (Balon, 1975), are commonly known to scatter their sticky eggs in the upper layer of shallow fast running gravel banks, where the eggs develop and the hatched larvae are drifted away (barbel: Hancock et al., 1976; nase: Peňáz, 1996; Ovidio & Philippart, 2008). Peňáz (1974) mentioned the refugial function of the interstitial for nase embryos first, Persat and Olivier (1995) described a below-gravel stage in the development of nase larvae and Schiemer et al. (2002) documented an emergence movement of nase larvae post-hatching. However, only recently the importance of the interstitial zone for the early life stages of this species was understood in greater detail, by detecting (a) that most of the eggs develop under the gravel surface and (b) post-hatching nase larvae move deeper into the interstitial zone (Duerregger et al., 2018). Consequently, the need for a better understanding of effects of different interstitial conditions on the development of the early life stages of nase is stressed, since, comparable to salmonid species, nase larvae depend on a loose and permeable stream bed in the embryonic phase to grow and increase swimming capacity before emergence. This is of even greater importance as populations are in a steep decline in their whole distribution area (Peňáz, 1996) and, especially in Bavaria (Germany), where this trend has not yet stopped (Mueller et al., 2018). Moreover, nase is an important component in the food web of riverine systems by regulating primary production by grazing benthic algae (Gerke et al., 2018), as well as providing a food source for higher trophic levels, such as the apex predator danube salmon (*Hucho hucho*, L.) (Šubjak, 2013). Therefore, this study focuses on a crucial bottleneck in the life cycle of nase, by analysing the effects of different sediment compositions on eggs and larvae under standardised conditions in experimental flumes in a laboratory. Such information on the autecological habitat requirements of this keystone species is essential in providing information for evidence-based restoration (Geist & Hawinks, 2016; Pander & Geist, 2013). Analogously to salmonid species (Sternecker & Geist, 2010), we hypothesised that substrate composition affects the (i) survival and the emergence rate, (ii) the timing of emergence and (iii) the larval length at emergence.

6.3   Material and Methods

6.3.1 Experimental setup

Modified salmonid egg incubation boxes and through-flow flumes were used to analyse the effect of different substrate types on development and emergence success (Figure 17). Salmonid egg incubation boxes (Sternecker & Geist, 2010) were modified for smaller eggs by lining it with a 500-µm plastic gauze. Each incubation box was subdivided into four individual compartments of equal size (L = 410 mm × W = 105 mm × D = 145 mm), each independent from the other, since water exchange and movement of larvae was prevented by separating boards (Figure 17).
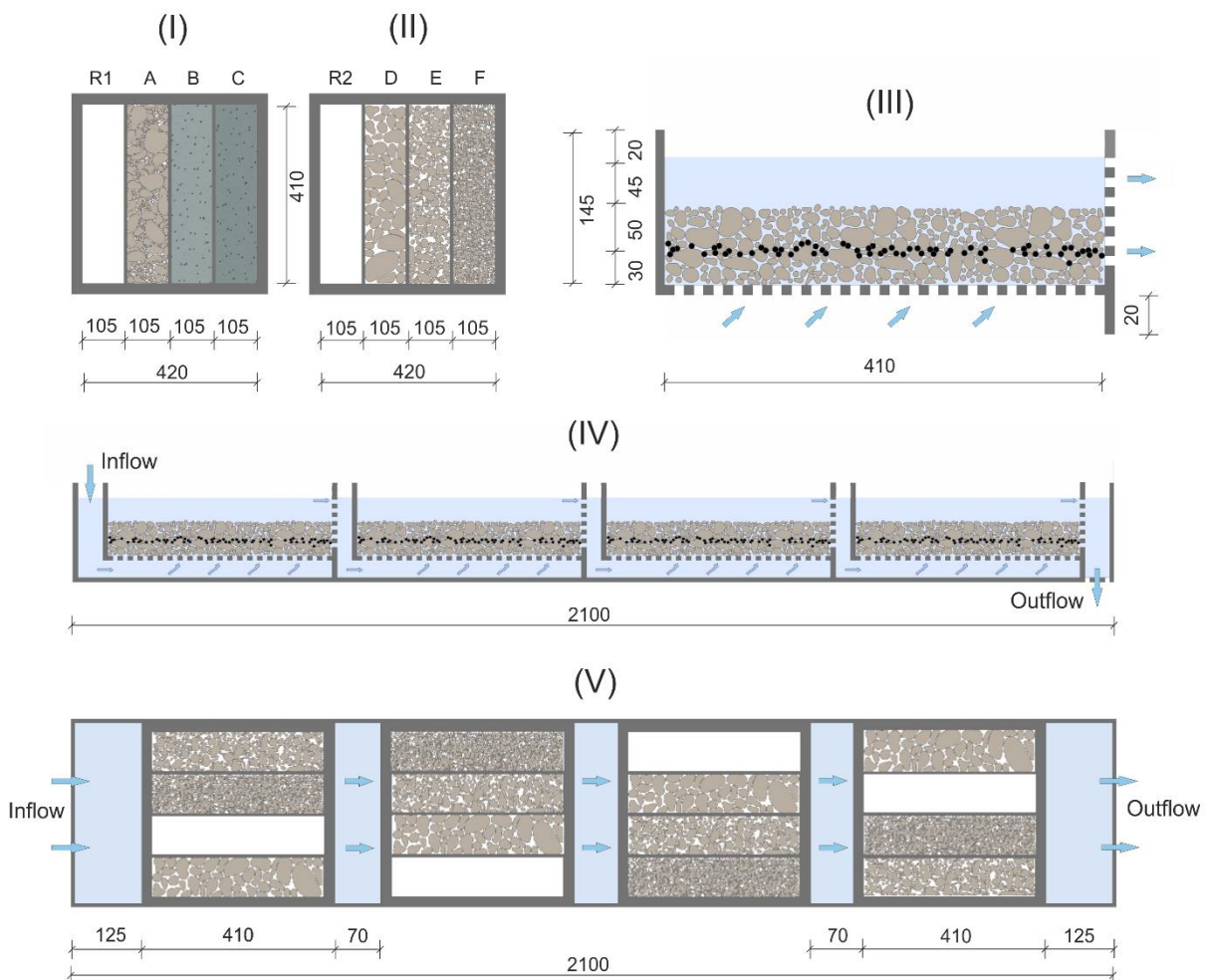
Figure 17: Schematic drawing of the experimental setup with dimensions (mm). (I and II) top view of the incubation boxes divided into four individual compartments of equal size (L: 410 mm, W: 105 mm and D: 145 mm); (III) side view of an incubation box with a 3 cm base layer of substrate at the bottom on which 150 fertilised nase eggs (black dots) are scattered, covered by another 5 cm layer of substrate; (IV) side view of four incubation

boxes placed in a laboratory flume; (V) top view of a laboratory flume with four boxes, each divided into four individual compartments with randomly placed treatments. Substrate composition followed Table 8

In half of the experimental plots (treatments A–C) different contents of fine sediment (<0.85 mm) were added to a basic substrate mixture (Figure 18 & Table 8). The second setup (treatments D–F) used defined substrate compositions of homogenous gravel size classes (Figure 18 & Table 8). For the different fine sediment additions (subsequently termed FINE-ADD), a substrate matrix reflecting naturally occurring conditions on functional spawning grounds of nase (Duerregger et al., 2018) was used as basic mixture (treatment A). To test for the effects of different fine sediment loads, two more treatments were set up, where 10% (treatment B) and 20% (treatment C) of additional fine sediment (<0.85 mm) were added to the basic mixture respectively (Figure 18 & Table 8).

To mimic natural condition, this fine sediment was directly collected on a natural spawning ground of nase (see Duerregger et al., 2018), containing <1.5% organic matter as determined by loss weight on ignition. For the different gravel fractions (subsequently termed GRAVFRAC) three gravel size classes similar to those commonly described for the restoration of spawning grounds were taken (Barlaup et al., 2008; Pander et al., 2015a; Pulg et al., 2013). All substrate used in the study was taken from the river Mangfall (12°6'23.52" east, 47°50'46.66" north), where several large and functional spawning grounds of nase are located (Duerregger et al., 2018). The collected substrate was wet sieved in the fractions of 20–63 mm, 6.3–20 mm, 2.0–6.3 mm, 0.85–2.0 mm and <0.85 mm and subsequently heat-treated for 24 hr at 100°C to eliminate potential bias from pathogens and predatory invertebrates. Before the start of the experiment, the substrate was acclimatised in hatchery water (groundwater supply) for 72 hr. For treatments A–C and D–F two through-flow flumes were used with a groundwater dotation of 0.1 Ls-1, each equipped with four incubation boxes, resulting in eight replicates per treatment. In each box, three treatments and one reference without substratum were randomly placed (Figure 17).

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Table 8: Percentages (% by weight) of substrate types use for each treatment

		20-63 mm	6.3-20 mm	2.0-6.3mm	0.85-2.0 mm	< 0.85 mm
Treatment	**A**	50.0	30.0	15.0	5.0	0.0
	B	45.0	27.0	13.5	4.5	10.0
	C	40.0	24.0	12.0	4.0	20.0
	D	100.0	0.0	0.0	0.0	0.0
	E	0.0	100.0	0.0	0.0	0.0
	F	0.0	0.0	100.0	0.0	0.0
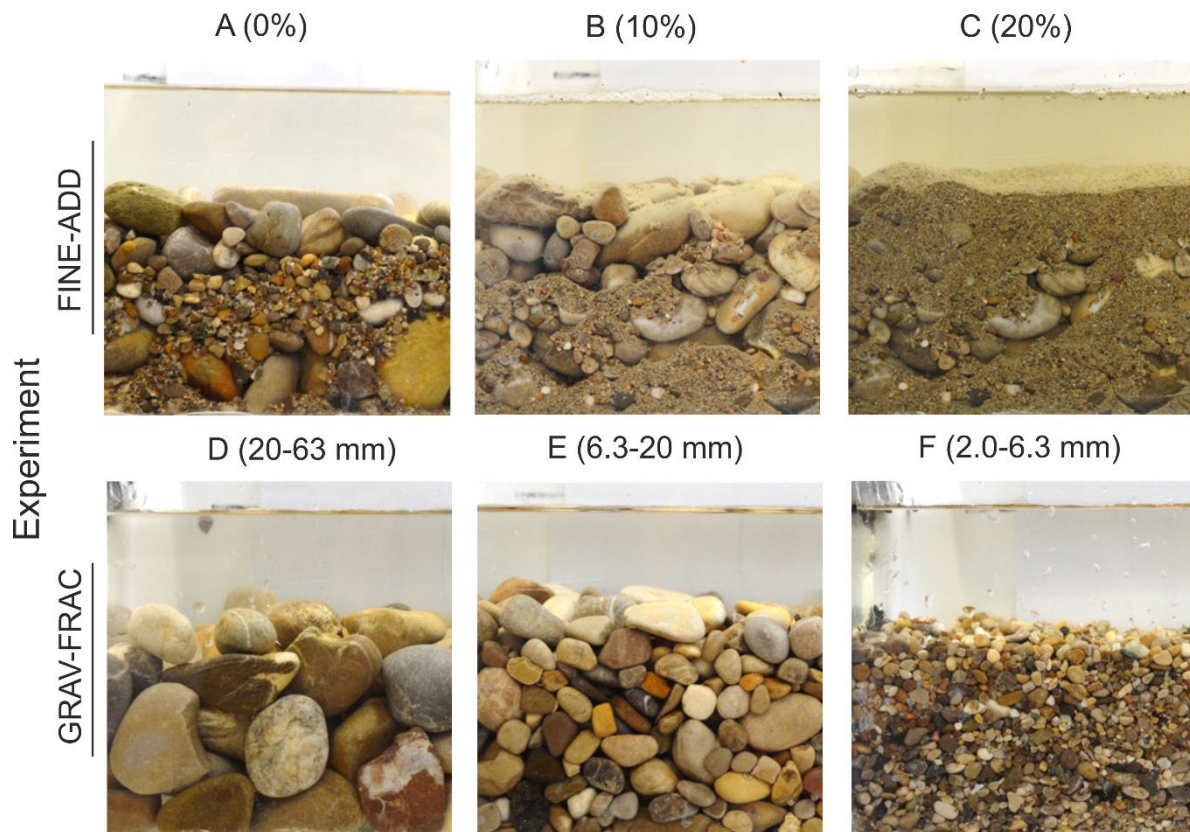
Figure 18: Photographs of the different substrate types used in this study. Substrate composition was mixed according to Table 8. FINE-ADD refers to treatments with different fine sediment additions; GRAV-FRAC refers to treatments with different homogenous gravel fractions

Eggs were collected from three females and fertilised by four males, caught by electrofishing during the spawning migration in the River Mangfall within the breeding programme of the local angling clubs. According to Halačka and Lusk (1995), the highest mortality in eggs of nase

occurs during the first three days of incubation. Therefore, the experiment was started 60 degree days after fertilisation by scattering 150 eggs on a base layer of 3 cm substratum in each compartment. Eggs were then carefully covered by another 5 cm of substratum with the same composition (Figure 17). Light conditions in the laboratory followed the natural day lengths. Every 24 hr, emerged larvae in the treatments and hatched larvae in the reference were collected by vacuum suction using a glass tube. The emerged larvae were counted and preserved in 96% ethanol for further analysis of total length. Three days after the last larvae emerged, the substratum was carefully removed and physically blocked larvae counted and preserved as well. All the work conducted was in compliance with the German animal welfare act.

6.3.2 Physicochemical measurements

Temperature was measured hourly using individual data loggers in each of the flumes (Lascar Electronics Ltd; www.lascarelectronics.com). Mean temperature was at 12.3 ± 0.3°C. These data were used to calculate the duration of emergence in degree days (dd). Degree days is a common parameter for describing the duration between fertilisation and hatching in fish species and is calculated by summing up the daily mean water temperatures. Measurements taken with a hand-held Multi 3,430 equipment and a pH3110 (WTW) in each flume at the beginning and at the end of the experiment revealed a mean oxygen concentration of 9.12 ± 0.3 mg/L, a mean electric conductance of 1,088 ± 73 µS/cm, a mean pH of 8.2 ± 0.1 and a mean redox potential of 518 ± 13 mV. Turbidity was measured using a PhotoFlex Turb hand-held field measurement unit (WTW) and revealed a mean of 1.8 ± 1 NTU. Additionally, photometric measurements using PhotoLab S12 (WTW) of nitrite (0.04 ± 0 mg/L), nitrate (1.08 ± 0.2 mg/L) and ammonium (0.04 ± 0.02 mg/L) were taken.

6.3.3 Determination of larval total length

Random subsamples of 30 emerged larvae from each treatment and each day were taken for determination of total lengths. On days when less than 30 larvae emerged from a treatment, all larvae were measured. Measurements of total length (± 0.1 mm) were taken using a stereo-

microscope Olympus SZX10 (Olympus Deutschland GmbH) with a magnification of 6.3 and the cellSens-Software (OLYMPUS CORPORATION; www.olympus-lifescience.com).

6.3.4 Data analysis

Total emergence rate (%) was calculated by adding up the daily emergence rate per treatment in relation to the total amount of eggs initially incubated. Adding the numbers of physically blocked larvae within the substrate found after terminating the experiment to the total emergence rate revealed the total hatching rate in each treatment. To compare the timing of emergence (E) between treatments and the hatching rate (H) in the reference the 0.25 (E25 and H25), 0.5 (E50 and H50) and 0.75 (E75 and H75) percentiles were calculated from the total emergence count and total amount of hatched larvae in the reference. Mean and standard deviation (*SD*) were calculated for the number of emerged larvae per day and the larvae length at emergence. Values are reported as means (± standard deviation) unless stated otherwise. Prior to the statistical significance tests, Shapiro–Wilk and Levene tests were applied to check for normal distribution and homogeneity of variances. Since none of the analysed data followed a normal distribution or homogeneity of variances, differences in the emergence count and the timing of emergence were tested with Kruskal–Wallis tests and post hoc Mann–Whitney U tests. The effect of substrate composition and incubation time on total length of larvae at emergence was tested using two different linear mixed effects models with the function "lmer" in the package "lme4" in R

(R Core Team, 2017). Firstly, to assess the effect of treatment on larvae length, the response variable larvae length at emergence was linked to the fixed factor treatment only. In order to also account for the factor time, a second model was constructed, in which the response variable larvae length at emergence was linked to the fixed factors treatment, time and the interaction term between treatment and time. In both models, compartment was set as a random effect. Pairwise comparisons between treatment levels were calculated using the function "glht" in the R package "multcomp", significances were assessed using a Wald chi-square test. *p*-values were adjusted with a holm correction. Diagnostic plots in R were used to visually evaluate the residuals of these fitted models in terms of normality of errors and homogeneity of variance. To visualise the interaction between larval length at emergence and incubation time within the different treatments the function "vis.reg" in the R package

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"vis.reg" was used. References R1 and R2 were combined for this analyses since they both reflect the same conditions (single compartments without substrate). All statistical analyses were performed using R (version 3.5.1, R Core Team, 2017). For all statistical analyses, significance levels were set to $p < .05$.

6.4 Results

6.4.1 Emergence success

From the standardised substratum mixtures with different fine sediment additions (FINE-ADD; treatments A–C), a total of 2,782 larvae (77% of exposed eggs) emerged successfully within 470 dd. From the three gravel fractions (GRAV-FRAC; treatments D–F), a total of 2,601 (72%) larvae successfully emerged within the same period. Substrate composition had a highly significant effect on the number of emerged larvae for both the fine sediment additions (Kruskal–Wallis Test: χ^2 = 18.385; *df* = 2; p < .001) and the gravel fractions (Kruskal–Wallis Test: χ^2 = 16.388; *df* = 2; p < .001). The number of emerged larvae was significantly higher in treatment A containing least fine sediment (emergence rate 98%), compared with treatments B (emergence rate 79%) and C with the highest fine sediment content (emergence rate 55%; pairwise Mann– Whitney U test: p < .01). The hatching rate in treatment A was 3% higher than the hatching rate in the reference without substrate (95%) (Figure 19a). The mean number of emerged larvae in the period from the first until the last emerged larvae per day counted was 84 ± 81 larvae in treatment A, 59 ± 65 in treatment B and 33 ± 39 in treatment C.

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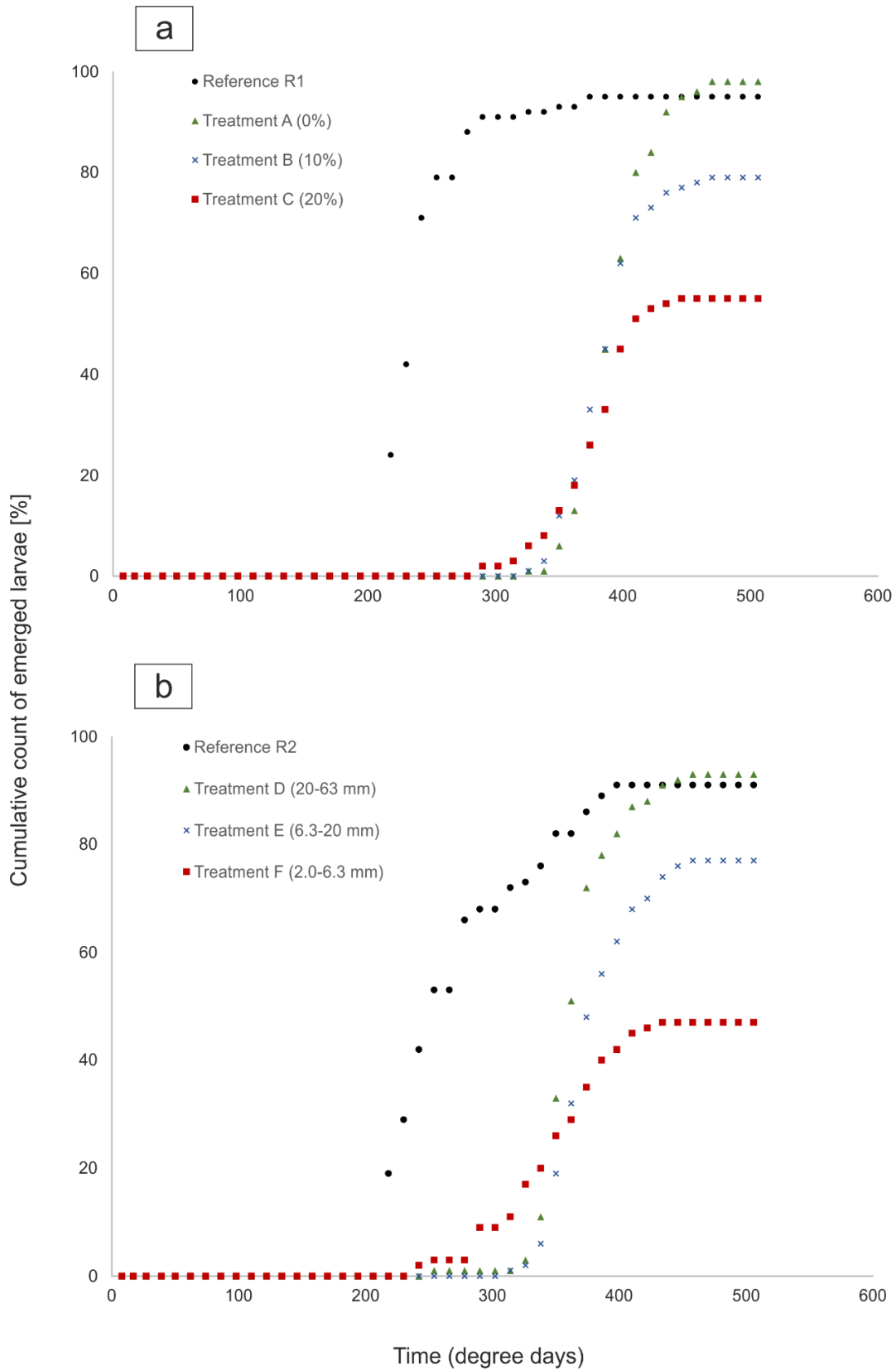
Figure 19: Chronology of cumulative emergence rate (%) for the three fines additions (FINE-ADD) treatments (A–C; a) and the three gravel size fractions (GRAV-FRAC) treatments (D–F; b). Substrate composition followed Table 8

A similar pattern was observed in the experiment concerning the different gravel fractions. The number of emerged larvae was significantly higher in treatment D with the coarsest grain size (emergence rate 93%) and treatment E (emergence rate 77%) compared with treatment F containing the smallest grain size (emergence rate 47%; pairwise Mann–Whitney U test: $p <$ .01). Equally to the fine sediment additions, treatment D showed an even higher total emergence rate (93%) compared with the hatching rate in the reference (91%) (Figure 19b). Mean emergence rate in the gravel fractions was 53 ± 83 larvae in treatment D, 49 ± 59 in treatment E and 27 ± 27 in treatment F. Three days after the last larvae emerged, the removal of the substratum revealed physically blocked larvae in every treatment (Figure 20). In the fine sediment additions, only a few individual larvae were found, with lowest numbers counted in treatment A (1.9 ± 1.5), followed by treatment C (2.4 ± 1.6) and treatment B (4.9 ± 4.8). The removal of the different gravel fractions revealed similar numbers of physically blocked larvae for treatment D (0.1 ± 0.3) and treatment E (1.8 ± 1.7), but a remarkable increase for treatment F (47.1 ± 17.9). Adding the numbers of living but physically blocked larvae (given in brackets) found in the substrate after termination of the experiment to the numbers of overall emerged larvae in each treatment during the course of the experiment, revealed the total survival rate of each treatment, which was 99 (+1)% for treatment A, 82 (+3)% for treatment B, 56 (+3)% for treatment C, 93 (+0)% for treatment D, 78 (+1)% for treatment E and 79 (+32)% for treatment F.
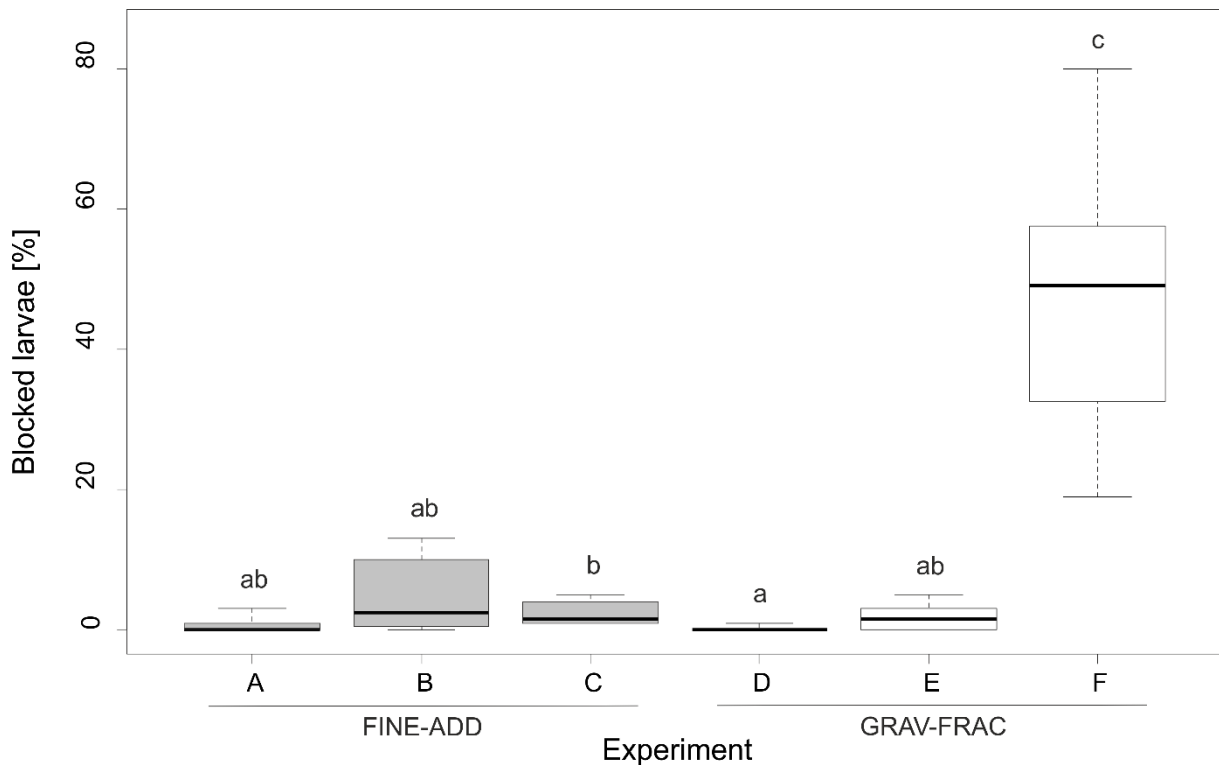
Figure 20: Number of blocked larvae found after termination of the experiment. Identical letters above boxes indicate statistically homogenous groups (n = 8)

6.4.2 Timing of Emergence

The timing pattern of emergence was similar throughout all treatments. In the beginning, a few individual larvae emerged, interrupted by days with no emergence at all. Subsequently, a distinct increase in emergence activity was observed, until the peak was reached at 374 dd and numbers began to decrease again until termination of the experiment after 506 dd. First hatching in the reference compartments of the FINE-ADD treatments occurred after 218 dd, whereby almost 80% hatched within 4 days (218–254 dd) (Figure 19a). In the following days, only scattered hatching took place and after 398 dd all larvae had hatched in the reference. Emergence activity started at 242 dd with two single emerged larvae in treatment C, but the overall peak in emergence was observed considerably later at 398 dd. Even though the time span increased by the content of fine sediment in the substrate, no significant differences in the median time of peak emergence in the treatments of the FINE-ADD setup were found (Figure 21). Remarkably, the H50 in the reference without substrate and the E50 in the respective treatments showed a significant delay of 144–156 dd in the treatments with different fine sediment additions (Figure 21).

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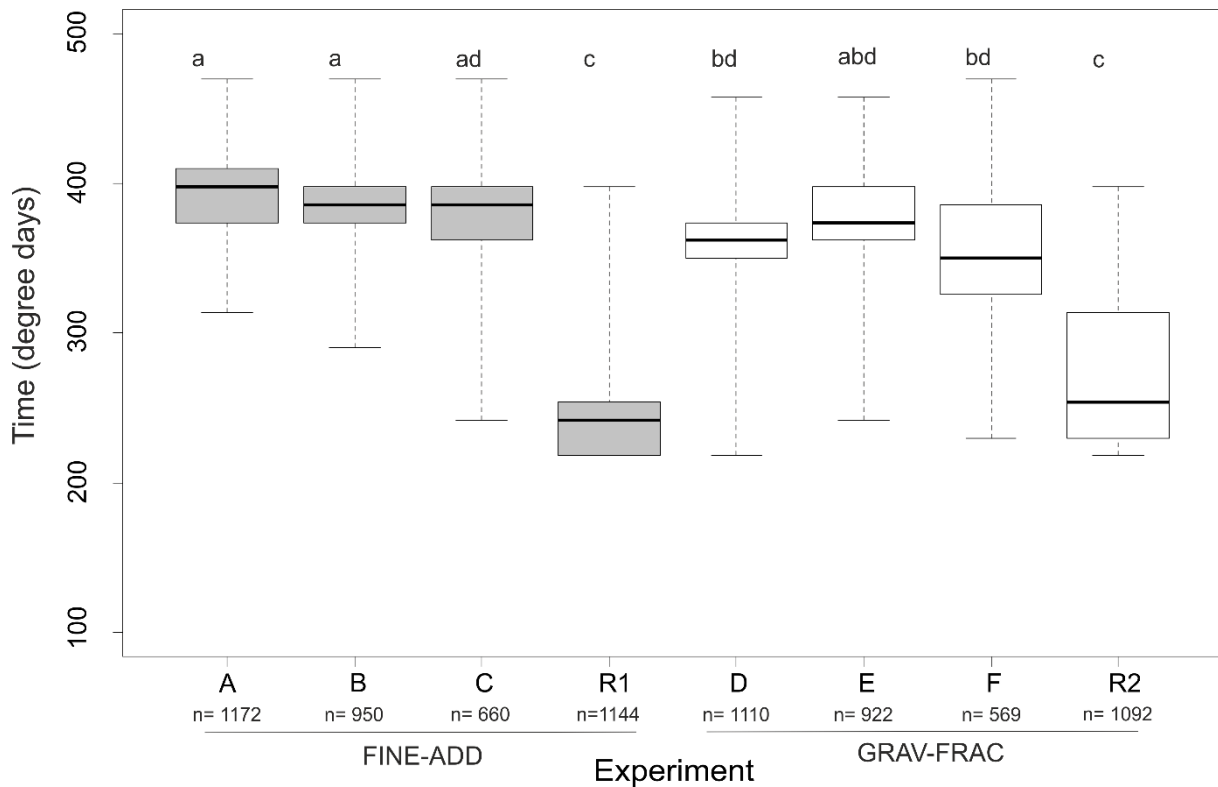
Figure 21: Emergence timing of the treatments with fines additions (grey) and gravel size fractions (white) in relation to day degrees. Substrate composition followed Table 8. Boxes: 0.75 percentile is conform to 75% (E75) of total emergence of each treatment; the 0.25 percentile is conform to 25% (E25) of total emergence of each treatment. The Median shows 50% of emerged larvae calculated on the total amount of emerged larvae of each treatment. Whiskers show the first and the last emerged larvae. Emergence in the treatments (A–D) corresponds to hatching (H) in the reference (R1 and R2). Identical letters above boxes indicate statistically homogenous groups based on median testing

In the reference, compartments of the GRAV-FRAC treatments hatching started at 218 dd and lasted until 398 dd. Within 4 days (218–254 dd), more than 50% of the reference larvae hatched, followed by decreasing numbers in the daily hatching rate (Figure 19b). Emergence in the GRAV-FRAC setup started at 218 dd with single emerged larvae in treatment D, whereas the overall peak in Treatments D–F was observed considerably later at 350 dd. No significant differences in the median time of peak emergence were observed, even though the time span of highest emergence activity (E25–E75) decreased by increasing gravel size (Figure 5). Similar to the treatments with different fine sediment additions, the treatments with different gravel fractions revealed a significant delay of 96–120 dd between the H50 in the reference without substrate and the E50 in the respective treatments (Figure 21).

6.4.3 Larval length at emergence

Over all treatments, total lengths of larvae at emergence ranged from 8.8 to 14.9 mm. Mean larvae length decreased with increasing amount of fine sediment and was highest in treatment A (13.4 ± 0.4), followed by treatment B (13.3 ± 0.6), treatment C (12.9 ± 0.8) and the respective reference R1 (11.7 ± 0.9). Generally, an increase in larvae length with time throughout the period of emergence was observed. This increase was dependent on the percentage of fines, with a steeper increase over time in treatments with elevated fine sediment content (Figure 22). However, when time was accounted for, larvae in treatment C were significantly smaller compared with treatment A (Model 2; $p < .05$), the latter characterised by the highest contentof fine sediment. In the defined gravel fractions, mean larvae length was highest in treatment E (13.3 ± 0.4), followed by treatment D (13.1 ± 0.8), treatment F (12.7 ± 0.8) and the respective reference R2 (12.2 ± 1.0). LMMs detected significant differences in the treatments A–E in comparison with the size of larvae at hatching in the reference without substrate (Supporting information 2). Taking into account the interaction between treatment and time, the factor treatment had no significant effect on larvae length ($\chi2(6) = 4.86$; $p = .56$), whilst the time ($\chi2(1) = 1,461.46$; $p < .001$) and the interaction of treatment and time ($\chi2(6) = 209.48$; $p < .001$) had significant effects on larvae length at emergence (Model 2).
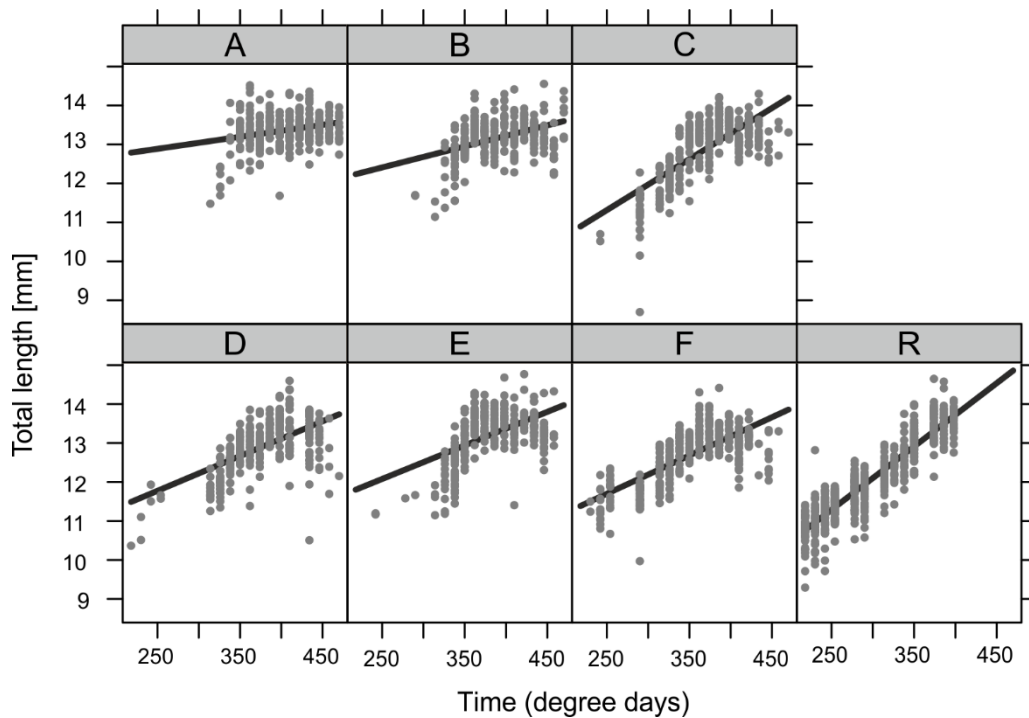
Figure 22: Scatter plot of larval size at emergence (treatments a–f) and larval size at hatching (r) in relation to incubation time. References R1 and R2 are combined in this Figure. Relationships between larval length and time are based on the interaction term of treatment and time within the linear mixed effects model and visualised using the R function vis.reg

6.5   Discussion

The results of the study demonstrate that grain sizes and sediment composition can impact on recruitment levels of nase by affecting emergence success, timing of emergence and size of larvae at emergence. This interpretation is supported by both experimental setups and highlights the importance of interstitial conditions for nase early life stages.

6.5.1 The effects of substrate on emergence and survival

A clear negative effect of fine sediments and small grain sizes on emergent survival of nase larvae was observed in this study. The results are in line with field observations in nase (Duerregger et al., 2018) and dace (*Leuciscus leuciscus, L.*) (Mills, 1981) where a negative impact of fine sediment infiltration on the development success of eggs on natural spawning grounds was demonstrated. They also match findings from studies on salmonids, which indicated an adverse effect of small grain sizes on the emergence and survival in a similar

laboratory setup (Sternecker & Geist, 2010) and in other experiments under laboratory conditions (e.g. Louhi et al., 2011; Phillips et al., 1975). The strongest effects of fine sediment are already evident in egg and larval development, which is the reason for the high mortality, derivable from emergence success as well as the low numbers of living but physically blocked larvae found at the end of the experiment in treatments with fine sediment addition (B & C). This is likely due to negative effects of fines not only on the emergence process, but on the egg development success as well, possibly through blocked diffusion of oxygen through the fine particles (Greig et al., 2005b). In a previous field study, oxygen has already been identified as a crucial parameter for successful egg development of nase (Keckeis et al., 1996). Moreover, the negative effect of fine sediments can be explained by reduced pore space and movement possibilities as well as by limited oxygen supply and accumulation of metabolic products (Crisp, 1996; Greig et al., 2005b). Larger gravel sizes tend to be relatively loosely packed, resulting in a higher porosity (Kemp et al., 2011). Therefore, movement of larvae as well as water exchange is much easier in this type of substrate. The high numbers of physically blocked larvae (32%) found in treatment F (2.0–6.3 mm) after termination of the experiment demonstrate that this grain size provides sufficient conditions for successful egg development, but form a physical barrier by limiting movement possibilities and thereby preventing hatched larvae from successful emergence. In a study on the emergence of salmonid fry by Sternecker and Geist (2010), a similar rate of physically blocked larvae (30%) was observed for brown trout (*Salmo trutta*, L.) in the grain size fraction 5–8 mm, indicating that a sole presence of these grain sizes seem to be a general problem for several lithophilic fish species, irrespective of different egg and larvae sizes. The importance of a loose and porous interstitial is evident for egg development and emergence of larvae likewise and is also supported by the high emergence rates in treatment A (0% fines) and treatment D (20–63 mm) that showed an even higher survival rate than the hatching rates in the respective reference without substratum, similar to field observations by Duerregger et al. (2018). This highlights the refugial function of a loose interstitial zone for egg and larvae development in contrast to eggs deposited on the gravel surface.

6.5.2 Timing of emergence

The timing of emergence in the substratum mixtures was significantly affected by the substrate composition, creating a higher variation in the timing of emergence, for example an increasing time span of emergence, by an increasing content of fines. Effects of sand in the spawning substrate have also been reported for barbel, where hatched larvae showed early emergence in treatments with elevated sand content (Bašić et al., 2018). Additionally, high fine sediment shares in spawning substrate might form a physical barrier that slow down the emergence process, which is evident by the high numbers of physically blocked larvae in treatment F (20% fines). A similar finding was reported in a study by Hausle and Coble (1976) where the chronology of emergence in brook trout alevins (*Salvelinus fontinalis*, R.) was delayed with increasing proportions of sand (<2 mm) in the redds. In all treatments, a distinct lag phase of up to 156 degree days between hatching and emergence was observed compared with the corresponding references. This shows that post-hatching free embryos of nase use the shelter of the interstitial zone to further develop and only emerge several days after hatching when the yolk sac is almost fully resorbed and the embryonic period is completed. A sheltered environment for the early ontogeny is of great importance since growth of nase larvae is strongly related to swimming capacity (Flore et al., 2001; Hofer & Kirchhofer, 1996; Schludermann et al., 2009), which increases the chance of survival, since poor swimmers face a greater risk of predation and uncontrolled downstream displacement (Louhi et al., 2011). This is also supported by field observations from Duerregger et al. (2018), showing a vertical movement of nase larvae post-hatching deeper in the interstitial zone. Larval movement in the pores of the interstitial zone has been also described for other litophilic cyprinids, such as vertical movements of the common minnow (*Phoxinus phoxinus*, L.) (Bless, 1992) and downstream interstitial movement of the barbel (Vilizzi & Copp, 2013). These findings, in combination with our results, highlight the importance of a functional permeable interstitial on spawning grounds of gravel spawners from the Cyprinidae family as eggs deposited in the interstices provide an immediate shelter for larvae post-hatching. On spawning grounds, where the interstitial is clogged and smothered by fines, eggs can only be deposited on the gravel surface, thereby exposing them and freshly hatched embryos to predation and uncontrolled off-drift (Keckeis et al., 1996; Persat & Olivier, 1995).

6.5.3 Larval length at emergence

Our results showed differences in the larvae lengths among the treatments with the size of larvae at the point of emergence decreasing with an increasing content of fines in the substrate. Consequently, fine sediment seems to not only directly affect mortality and emergence success but also more subtle endpoints, such as growth, that may be of evolutionary importance. The relevance of size-selective effects has to date been mostly discussed in the context of fisheries-induced evolution where removal of larger elements of a population has been linked with retarded growth and lower biomass yield in following generations (e.g. Conover & Munch, 2002). The observed effects of smaller larvae sizes at increasing amounts of fines in this study on nase and a previous study on salmonids (Sternecker & Geist, 2010) point in a similar direction, yet it remains to be tested if they are inheritable and if there is compensatory growth when fish become older. The smallest larvae were observed in treatments with highest fine sediment addition (20%; treatment C) and the smallest gravel fraction (2.0–6.3 mm; treatment F). Both the high numbers of physically blocked larvae in treatment F and the high mortality in treatment C suggest the possible existence of a size selection of hatched larvae on spawning grounds with small gravel sizes or high content of fine sediment. On the other hand, the smaller sizes may also be explained by impaired growth at adverse interstitial conditions of larvae developing in an interstitial where pores are clogged by fines (e.g. treatments B & C) or grain sizes are consistently small (e.g. treatment F) in contrast to larval development in a substratum with higher porosity (e.g. treatment A) and greater pore sizes (e.g. treatment D & E). In any case, reduced growth leads to an extended development phase of these critical life stages and thereby increases the risk of mortality (Schiemer et al., 2002). This is of great importance since growth is directly related to survival, especially during the early ontogeny of fish (Bolland et al., 2007; Osse et al., 1997). Observations on the vertical distribution of nase eggs on spawning grounds in the field revealed eggs down to 12 cm (Keckeis et al., 1996) and 30 cm in the substrate (Duerregger et al., 2018). In this context, our results can be seen as a conservative approach in contrast to naturally occurring conditions as in this study the substrate layer through which larvae had to emerge was set to only 5 cm. Therefore, the need for a loose interstitial zone for nase larvae must be stressed as it apparently contributes positively to the embryonic development and thereby to successful recruitment of this species. Moreover, we observed an increase of larvae

length with an ongoing period of emergence, indicating that growth is not concurrent for all larvae. Larvae start to emerge as soon as they reach a certain development stage, reflected by their total length. Therefore, it is likely that under ideal conditions only larvae of a similar development stage show emergence activity (as evident for treatment A), mainly driven by resorption of the yolk sac and the onset of exogenous feeding (Persat & Olivier, 1995). Although a linear increase in growth of larvae post-hatching has been described for several fish species and for nase in particular (Limburg, 1996; Otterå, 1993; Schludermann et al., 2009), larvae length in substrate textures with no or little content of fines (treatment A & B) showed almost no increase in total length over time at all. This pattern is not evident to the reference treatments without substrate, where larvae were counted as they hatched. In substrata with insufficient development conditions (e.g. high contents of fine sediment or small pore sizes) larvae possibly try to escape from these adverse effects, irrespective of size and development stage. Since the blocking effect of these substrates form a physical barrier and thereby slow down the emergence process, this ultimately results in an extended time span in the development phase and the period of emergence likewise.

6.6   Conservation implications

Our results highlight the importance of the quality of the interstitial zone for nase embryos as survival, emergence success and size is severely influenced by the substrate composition, in particular by the content of fine sediment. This shifts the focus on the improvement of the interstitial zone since anthropogenic impacts in European rivers often limit the potential for natural restoration of this key habitat. Restoration can include long-term measures, such as re-establishing dynamic flow processes in lateral and longitudinal direction and thereby sediment dynamics (Auerswald & Geist, 2018) as well as reduction of fine sediment infiltration in rivers due to erosion by land use (Davies et al., 2009; Knott et al., 2018) and short-term restoration measures, such as gravel cleaning (e.g. Bašić et al., 2017; Shackle et al., 1999) and gravel introduction (e.g. Pander et al., 2015a; Pulg et al., 2013). Moreover, we discovered a distinct lag phase in the timing of hatching and emergence, thereby proving that nase larvae use the interstitial zone as a sheltered environment for development post-hatching. It is evident that a loose and permeable interstitial can fulfil this role much better than a colmated streambed. This is of evolutionary importance since embryonic development poses an important environmental bottleneck for successful recruitment of nase populations. Therefore, we suggest integrating substrate quality in future assessments of nase spawning grounds as it has been common practice for salmonid species for a long time (e.g. Lotspeich & Everest, 1981; Sternecker et al., 2013a). The same holds true in conservation and restoration management of nase where the role of functional stream beds has to date not been sufficiently considered.

7. Making up the bed: Gravel cleaning as a contribution to nase (*Chondrostoma nasus* L.) spawning and recruitment success

A similar version of this chapter was published in:

Candidate's contribution:

Study design and methodology was developed by CN with constant input from MM, JP and JG. CN conducted all fieldwork. Data preparation and statistical analysis, including all figures and tables, were conducted by CN. The original draft was written and finalised by CN. Revision and editing of the article were done by CN, MM, JP and JG.

7.1   Abstract

Spawning substrate quality is a major factor influencing the early ontogeny of European nase (Chondrostoma nasus), a target species of conservation. Analogous to findings from salmonids, restoration of spawning grounds was hypothesised to enhance spawning, development and thus recruitment success of nase, by improving the substrate quality, and subsequently spawning site use, egg infiltration and protection of larvae in the interstitial zones before emergence. These assumptions were tested using a comparative approach by cleaning 50% of the area of each spawning ground in two Bavarian rivers. Substrate cleaning resulted in an immediate reduction of ~70% fine sediment content with improvements still detectable 2 months later. Spawning nase used the restored areas of spawning grounds preferentially, which was evident in the number of spawning fish and the significantly higher number of eggs laid. Infiltration of eggs into the interstitial zone was distinctly more successful

in the opened interstices of the cleaned spawning substrate, where they were found down to a depth of 20 cm. The same was true for larvae, which could be found down to 30 cm and up to 13 days after hatching. Moreover, higher peaks in the drift density of emerging larvae from the restored spawning substrate were detected (2.5 compared with 1.7 larvae m−3 discharge for the River Mangfall and 0.3 compared with 0.03 larvae m−3 for the River Sims). These results clearly indicate that gravel cleaning is a successful short-term restoration tool for nase spawning grounds. It is a quick, cheap and effective method for the conservation management of nase, which may also be applicable to other riverine species with a similar ecology and incubation time, such as Barbus barbus, Squalius cephalus, Leuciscus leuciscus and Phoxinus phoxinus. This especially holds true if streams lack internal dynamics and suffer from high loads of fine sediment and colmation.

7.2   Introduction

Habitat quality is a major factor in the survival of fish populations (Lapointe et al., 2013) and its widespread degradation has been identified as the main cause for the slow recovery of fish fauna in large rivers in Europe (Aarts et al., 2004). This is primarily evident in the functionality of gravel banks used as spawning grounds by lithophilic fish species, which is often reduced owing to fine sediment infiltration from erosion by land use and animal activity (e.g. livestock, crayfish burrowing; Davies et al., 2009) or restricted internal stream sediment dynamics (Auerswald & Geist, 2018). Excessive amounts of fine sediment introduction into the stream bed cause adverse effects by physically clogging substrate porosity (Geist & Auerswald, 2007) and through biogeochemical processes owing to the reduced oxygen supply (Greig et al., 2005a; Sear et al., 2017), which ultimately affect egg survival, hatching and the emergence of larvae (Jensen et al., 2009; Kemp et al., 2011; Sternecker & Geist, 2010). Consequently, the reproductive success of lithophilic fish species is often impaired. Synergistic effects between fine-sediment ingression and increased temperature explain greater susceptibilities of spring-spawning species compared with winter spawners (water temperature-related processes; Sternecker et al., 2014).

To date, studies analysing substrate-related effects and stream-bed restoration success on egg and larval survival have mainly focused on economically important salmonids (Kondolf, 2000; Phillips et al., 1975; Pulg et al., 2013; Soulsby et al., 2001; Sternecker et al., 2013a). Surprisingly, this topic has rarely been considered in lithophilic cyprinids, which also comprise species such as nase, barbel (*Barbus barbus* L.), chub (*Squalius cephalus* L.), dace (*Leuciscus leuciscus* L.) and common minnow (*Phoxinus phoxinus* L.), that have declined in recent decades, especially in Bavaria, Germany (Mueller et al., 2018; Pander & Geist, 2018). From this group, the European nase (subsequently referred to as 'nase') is threatened throughout its entire native distribution area (Peňáz, 1996), ranging from Central Europe north of the Alps to Eastern Europe in the basins of the Black Sea, southern Baltic Sea and southern North Sea (Kottelat & Freyhof, 2007). Although *Chondrostoma nasus* does not receive any special conservation recognition in the European Habitats Directive (Council of the European Communities, 1992), five other species of the genus *Chondrostoma* are listed in Annex II, requiring Member States to designate special areas of conservation for important sites where these species occur. However, severe declines of nase have occurred locally, which is evident in Bavaria where nase is listed as 'endangered' according to the Red List (Bohl et al., 2003).

Owing to its complex life cycle, which depends on the availability, quality and connectivity of various habitats for the different life stages, nase populations are threatened by river damming and channelisation and the related consequences of blocked migration routes and degraded habitats, especially spawning grounds (Ovidio & Philippart, 2008; Peňáz, 1996). Therefore, nase has become a target species for conservation in Central European rivers (Schiemer et al., 2002). Successful spawning of nase is divided into four crucial steps. First, spawning grounds need to be accessible. Nase is known to be a potamodromus species, aggregating in dense swarms to migrate in spawning runs from rivers to tributaries (De Leeuw & Winter, 2008; Melcher & Schmutz, 2010; Rakowitz et al., 2008). Consequently, river damming often hinders the accessibility of functional spawning grounds (Aarts et al., 2004). Second, hydromorphological conditions at spawning grounds need to meet their spawning habitat requirements. Nase prefer shallow riffles with fast currents for egg deposition (e.g. Melcher & Schmutz, 2010), requiring stable conditions throughout the period of egg incubation (Hauer et al., 2007). In addition, these riffles need to be in the immediate vicinity of pools as, apart from the spawning event itself, nase show a spatial separation of sexes

during the days of spawning, with females resting in the pools while males maintain position on the spawning grounds (Peňáz, 1996). Third, after spawning, eggs need to develop successfully on the spawning grounds. Recent findings suggest that most eggs develop under the gravel surface in the interstitial zone (Duerregger et al., 2018), providing a protected environment. In contrast, deposition of eggs on the gravel surface exposes them to predation and uncontrolled drift (Keckeis et al., 1996; Persat & Olivier, 1995). Finally, hatched larvae need to emerge successfully from spawning gravel, so infiltration of fines into the stream bed during early ontogeny can reduce emergence success and affect subtle endpoints, such as larval size at emergence (Nagel et al., 2020a).

Measurements to improve spawning success by restoration of spawning grounds have been widely discussed in the scientific literature (Taylor et al., 2019) and are used to support fish populations by many organisations such as local fishing associations and regulatory authorities. Primary target species mostly comprise salmonids such as brown trout (*Salmo trutta* L.; Pulg et al., 2013; Sternecker et al., 2013b; Zeh & Dönni, 1994), European grayling (*Thymallus thymallus* L.; Zeh & Dönni, 1994) and salmon (Cramer, 2012; Mih & Bailey, 1981). Implemented and recommended measures for restoration include cleaning of colmated gravel, e.g. using an excavator (Pander et al., 2015a; Pulg et al., 2013) or jetting lance (Bašić, et al., 2017) and addition of gravel of various size classes (e.g. Barlaup et al., 2008; Cramer, 2012; Pander et al., 2015a) as well as structural improvements of the stream bed using current deflectors or boulders (Gore et al., 1998; Gortz, 1998; Pander et al., 2015a). To date, little is known on whether the results from these studies are also applicable to lithophilic cyprinids. Therefore, there is a great need to extend knowledge on the interaction of spawning substrate and reproduction success to those species, as an increasing number of studies, mainly conducted under laboratory conditions, have demonstrated the effects of spawning substrate composition on the early life stages of these species, e.g. on the interstitial movement (Vilizzi & Copp, 2013) and emergence timing (Bašić et al., 2018) of European barbel, as well as hatching and emergence success of nase (Duerregger et al., 2018; Nagel et al., 2020a). Moreover, as the success of stream bed restoration in salmonids is often limited by the short duration of the effects (Mueller et al., 2014), these measures may be more effective for cyprinid species with a shorter egg incubation period. Consequently, the aim of this study was to test how the restoration of nase spawning grounds influences (i) the fine sediment content

of spawning substrates, (ii) spawning site use, (iii) infiltration of eggs and larvae into the interstitial zone and (iv) development and timing of emergence, as measured by the numbers and size of downstream drifting larvae. Specifically, we hypothesised that restoring the spawning grounds will result in a reduced fine sediment content in the spawning substrates, increased habitat use by the spawners and a greater depth range of eggs and larvae within the interstitial zone and subsequent differences in their development and timing of emergence.

7.3   Material and Methods

7.3.1 Study area

To test for the effects of substrate cleaning on the reproduction and recruitment of nase, two spawning areas with well-known morphology (see Duerregger et al., 2018) in the River Mangfall (12°6'23.52'' E; 47°50'46.66'' N) and the River Sims (12°9'1.02'' E; 47°51'4.20'' N) – two tributaries of the River Inn in Bavaria (Germany) – were chosen for investigation (Figure 23). These sites are among the most important known spawning grounds in the entire catchment area of the River Inn according to observations on the size of spawning populations in previous years. The Mangfall has a river length of 58 km, draining a catchment area of 1,099 km2 before it discharges into the River Inn at about 3.3 km downstream of the investigated spawning ground. The mean annual flow of the Mangfall is 17.6 m^3 s^{-1}, but as the investigated spawning ground is located in a diverted river section, discharge at this site is relatively stable at 1.5 m^3 s^{-1} throughout the year (www.hnd-bayern.de). The Mangfall is assigned as a 'heavily modified water body' with a moderate to poor ecological potential (www.umweltatlas.bayern.de) according to the European Water Framework Directive (Council of the European Communities, 2000). The Sims has a river length of 8 km, draining a catchment area of 94 km2. Directly downstream of the investigated spawning ground, the Sims meets the River Rohrdorfer Ache before it also discharges into the River Inn. Mean annual flow of the Sims is at 1.89 m^3 s^{-1} (www.hnd-bayern.de). The ecological status for the Sims is assessed as 'moderate' (www.umweltatlas.bayern.de). In both rivers, their hydromorphological dynamics are restricted owing to controlled discharge by weirs located

upstream of the spawning grounds, resulting in fine sediment (<0.85 mm) contents above 10%.
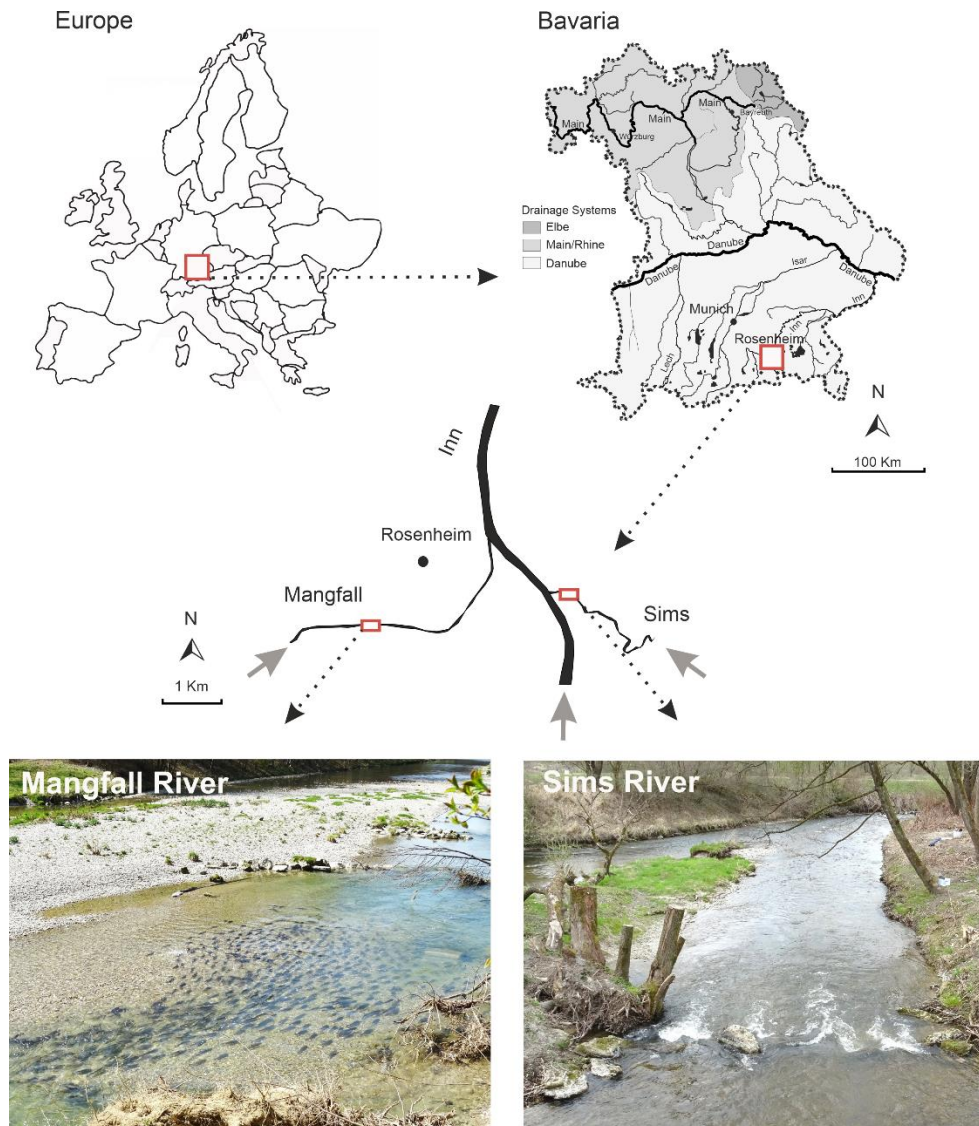
Figure 23: Map and photographs of the study area; grey arrows indicate flow direction. Note the spawning nase on the photograph of the River Mangfall

7.3.2 Abiotic measurements

Hydromorphological conditions in the spawning grounds were characterised by measuring water depth (cm) and current velocity (m s^{-1}) 10 cm above the substrate and 10 cm below the water surface in close proximity to each spawning box (see Section 2.5) on the day of installation. Importantly, no significant differences in the restored and the untreated site of each spawning ground were detected (Table 9). At the same points redox potential (mV) at 10

cm substrate depth was measured in situ, as described by Geist and Auerswald (2007) 6 days after the first spawning event using a hand-held pH 3110 meter (WTW, Weilheim, Germany). Measurements were also made of oxygen concentration (mg L^{-1}), pH, electric conductance (µS cm^{-1}) (with a hand-held Multi 3430; WTW, Weilheim, Germany) as well as turbidity (NTU) (using a hand-held PhotoFlex Turb equipment; WTW, Weilheim, Germany) each time drift nets were placed. Temperature was measured hourly using data loggers in each river (Lascar Electronics Ltd; www.lascarelectronics.com). These data were used to calculate degree-days (dd), by multiplying the mean daily water temperature with the days of incubation.

Table 9: Abiotic parameters of the investigated spawning grounds

	Mangfall		Sims	
	restored	**untreated**	**restored**	**untreated**
Flow velocity surface [m s^{-1}]	1.0 ± 0.2[a]	0.9 ± 0.3[a]	1.0 ± 0.1[a]	0.9 ± 0.1[a]
Flow velocity stream bed [m s^{-1}]	1.0 ± 0.1[a]	1.0 ± 0.3[a]	0.5 ± 0.1[b]	0.5 ± 0.1[b]
Water depth [cm]	30.2 ± 3.4[a]	27.9 ± 3.4[a]	47.1 ± 5.2[b]	42.9 ± 3.4[b]
Redox potential interstitial	473 ± 24[ab]	478 ± 16[a]	467 ± 10[ab]	447 ± 19[b]
O$_2$ [mg L^{-1}]	11.2 ± 1.0[a]		10.1 ± 1.0[b]	
pH	8.6 ± 0.2[a]		8.8 ± 0.2[a]	
Electric conductance [µS cm^{-1}]	485 ± 43[a]		453 ± 30[b]	
Water temperature [°C]	13.3 ± 3.0[a]		13.7 ± 3.2[a]	
Turbidity [NTU]	1.7 ± 0.6[a]		6.9 ± 2.4[b]	

Note: Values are given as means ± SD. Lower case letters indicate statistical differences between the restored and untreated sites of the spawning grounds in both rivers (regarding flow velocity, water depth and interstitial redox) and the water physico-chemistry of the two investigated rivers respectively

7.3.3 Fine sediment content of spawning substrate

Initial substrate quality was assessed with three freeze-cores at each site before restoration. At this time, fine sediment content (<0.85 mm) of spawning substrates was similar in both halves of the spawning grounds in the River Mangfall (treatment site, 12.2 ± 2.7%; control site, 10.9 ± 1.0%) and in the River Sims (treatment site, 10.1 ± 1.0%; control site, 9.2 ± 1.3%).

Another three freeze-cores at each site were taken 1 day after the restoration to account for changes in the substrate composition related to restoration. Subsequently, this sampling procedure was repeated ~40 and ~60 days after the restoration. Sediment samples from freeze-cores were wet-sieved in the fractions of >20–63, >6.3–20, >2.0–6.3, >0.85–2.0 and ≤0.85 mm using an electronic sieving-tower (Fritsch, Idar-Oberstein, Germany). Afterwards, discrete fractions were dried and weighed to determine percentages by mass.

7.3.4 Restoration of spawning grounds

Substrate restoration took place at the beginning of March, 5 weeks before the expected spawning runs of nase (Figure 24), as other studies showed that gravel cleaning is only effective for short periods if streams transport high loads of fine sediment (Meyer et al., 2008; Pander et al., 2015a).
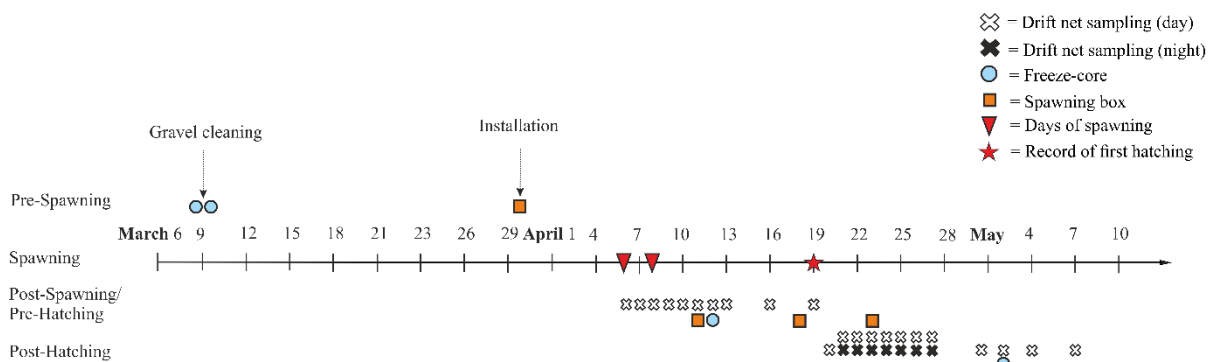
Figure 24: An example of the timeline of the experimental setup in the River Mangfall

Spawning grounds were divided lengthways into two parts of equal size, in which only one part was randomly selected for restoration while the other was left untreated to serve as a reference for naturally occurring conditions (Figure 25). This resulted in four investigated sites in total: Mangfall restored, Mangfall untreated, Sims restored and Sims untreated. Subsequently, the substrate of one half of each spawning ground was loosened and cleaned down to a depth of 50 cm by an excavator following the approach of Pulg et al. (2013) and Sternecker et al. (2013b).

(a)

(b)

(c)

(d)
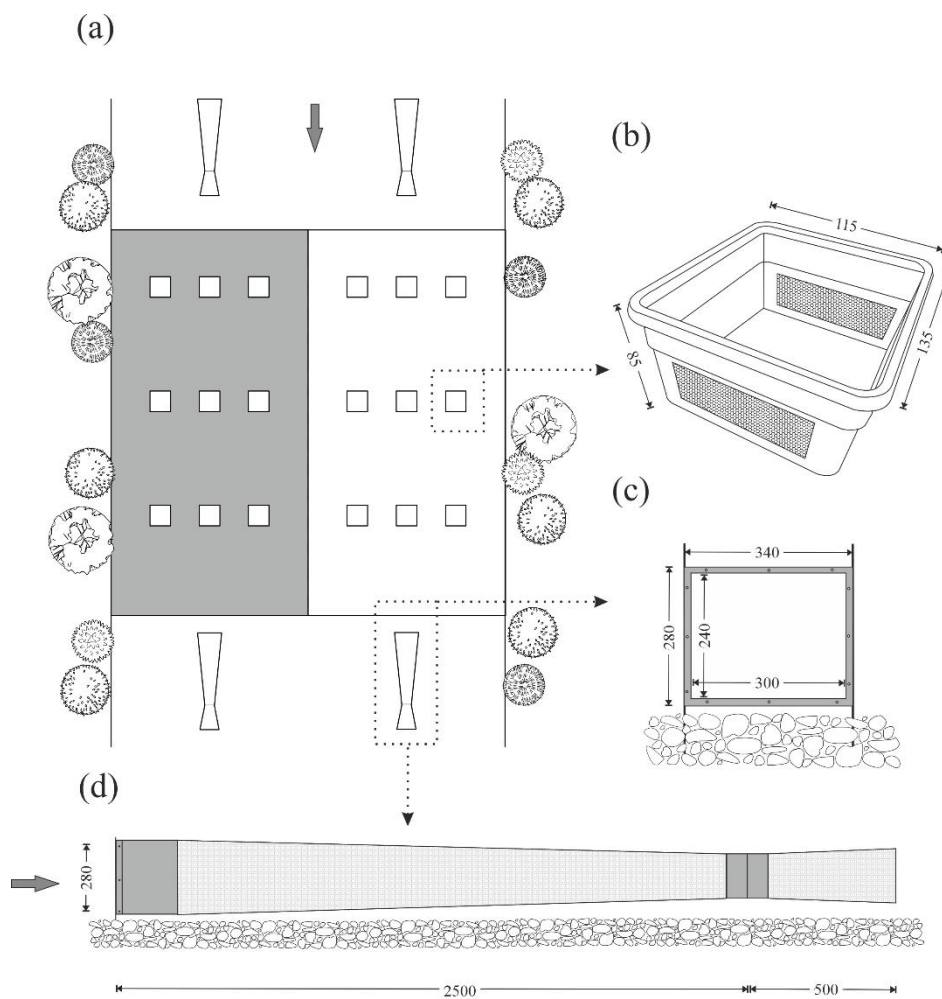
Figure 25: Schematic overview of the experimental setup on investigated spawning grounds (a). The grey part refers to the untreated site and the white part to the restored one. Squares show the positioning of the spawning boxes (b); trapeze-shaped symbols show the position of the drift nets (c and d). Dimensions are given in millimeters. Grey arrows indicate flow direction

7.3.5 Spawning site use

To evaluate substrate-related preferences in the use of spawning sites visual observations were conducted on the days of spawning counting the numbers of spawners per area. In addition, to quantify the number of eggs deposited immediately after spawning, as well as to estimate the decrease in the number of eggs at nase spawning grounds over incubation time, plastic boxes ('spawning boxes'; 16.5 cm × 14.5 cm × 8.5 cm, ROTHO clear boxes, ROTHO Kunststoff AG, Würenlingen, Switzerland) were used (Figure 25). Spawning boxes were comparable with those used in Duerregger et al. (2018), but of smaller size to limit the disturbance of the interstitial to a minimum. One week before the expected spawning event,

determined by gathering nase in further downstream areas, nine spawning boxes were equally distributed in each half of the spawning grounds, resulting in a total of 36 spawning boxes (Figures 24 and 25). Spawning boxes were retrieved every 5–7 days after spawning by randomly removing three boxes from the restored site in the spawning grounds and three boxes with corresponding positions from the untreated sites (Figure 24). Larvae had already started hatching by the scheduled date for the third retrieving interval, which is why only six out of nine boxes were assessed per treatment and river.

7.3.6 Infiltration of eggs and larvae into the interstitial zone

To quantify the horizontal distribution of eggs in the interstitial zone, three 30 cm deep freeze-cores equally distributed from downstream to upstream in each half of the spawning grounds were taken 6 days after spawning. Subsequently, freeze-cores were defrosted in layers of 10 cm and separately checked for eggs. The same method was applied 26 days after the first spawning event to check for remaining larvae in the interstitial zone. Sediment samples from freeze-cores were also used to evaluate changes in the substrate composition.

7.3.7 Development and timing of emergence

To evaluate the number of eggs that were not capable of entering the interstices of the stream bed, drift nets located downstream and upstream of each half of the spawning grounds were used, the latter to check for potential bias from spawning activity upstream of the investigated sites (Figure 25). Sampling devices were constructed using rectangular aluminium frames for the mouth (30 × 24 cm) and tear-proof polyester (mesh size ~800 μm) for the nets. The same method was applied to measure the timing of emerging larvae after spawning. To determine the amount of water filtered by every sampling device, flow velocity was measured six times in each frame (three at the upper end and three at the lower end) using an electromagnetic flow meter (Ott MF pro, Ott, Kempten, Germany) each time drift nets were set. Drift-net sampling started the day on which nase arrived at the spawning grounds and was continued on the following days for 1 h each day in the period from 12.00 to 17.00 (Figure 24). Two days after the first hatched larvae were caught, drift-net sampling was additionally conducted in

the hour between dusk and darkness (20:30–21:30) to check for light-dependent emergence patterns of larvae, as results of Persat and Olivier (1995) demonstrated that drift of nase larvae under experimental conditions is highest in the 2 h after dusk. This sampling schedule was maintained for 1 week and then changed back to only daylight sampling for one more week (Figure 24). Collected larvae were then preserved in 96% ethanol to determine total length (±0.1 mm) of all morphologically intact larvae using a stereo-microscope Olympus SZX10 (Olympus Deutschland GmbH, Hamburg, Germany) with a magnification of 6.3 and the cellSens-Software (Olympus Corporation; www.olympus-lifescience.com).

7.3.8 Data analysis

To quantify the fine sediment content of spawning substrate, cumulative texture lines were computed. In addition, arithmetic means (± standard deviation) were calculated for the fine sediment content of each investigated site at each date of sampling. Preferences in spawning-site use were tested with each spatially independent spawning box as a replicate (Supporting information 3). As these data did not follow a normal distribution and homogeneity of variances, differences in the egg count were tested for each river separately with Kruskal–Wallis tests. To assess the infiltration of eggs and larvae into the interstitial zone, the numbers of eggs and larvae in each substrate horizon are presented as arithmetic means (± standard deviation). This holds true for all values given in this study unless stated otherwise.

Drift densities (number of eggs or larvae caught in 1 m³ of filtered water) were calculated separately for each drift sampling device. In some drift samples from the River Sims, nase eggs and larvae were also found in the reference nets located upstream of the spawning ground. In this case, drift densities from reference nets were subtracted from drift densities downstream of the investigated spawning ground. Differences in the density of drifting eggs and larvae were tested for each river separately using linear mixed effect models (LMMs) with the function 'lmer' in the package 'lme4' in R (R Core Team, 2017). First, the response variable 'egg drift density' was linked to the fixed factor 'treatment' only. Second, to account also for daylight-depending patterns in the emergence of larvae, the response variable 'larval drift density' was linked to fixed factors 'treatment' and 'daylight' (day or night sampling). In all

models sampling date (days after first spawning for eggs, days after first hatching for larvae) was set as a random effect to account for temporal correlation between measurements.

Linear mixed effect models were also used to test for significant effects of treatment, river and daylight on the size of emerging larvae, as model assumptions regarding normal distribution of model residuals and homogeneity of variances were met (Supporting information 3). To account also for the interaction effects of treatment and river a second model was computed, in which the response variable 'larval length at emergence' was additionally linked to the interaction terms of these predictors. For both models, sampling date (days after first hatching) was set as a random effect. Model fit was assessed using standard graphical validation for LMMs in R (Zuur et al., 2009). The significance of effects was tested using a Wald $\chi 2$ test in the R 'car' package (Fox & Weisberg, 2011).

Distribution of larval length over time was visualised in weighted scatter plots using the function 'geom_count' from the package 'ggplot2' in R (R Core Team, 2017). All statistical analyses were performed using R (version 3.5.1, R Core Team, 2017). Significance levels were set to $p < 0.05$ for all statistical analysis.

7. Making up the bed: Gravel cleaning as a contribution to nase (Chondrostoma nasus L.) spawning and recruitment success

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7.4   Results

7.4.1 Fine sediment content of spawning substrate

Freeze-core samples taken before and one day after the restoration revealed an immediate reduction of fines (<0.85 mm) from 12.2 (± 2.6)% (w/w) to 3.6 (± 1.6)% in the River Mangfall (Figure 26a, c) and from 10.1 (± 1.0)% to 2.7 (± 0.6)% in the River Sims (Figure 4b, d). Freeze-core samples taken ~40 days after gravel cleaning showed a further decline of fines in the River Sims (2.1 ± 0.6%; Figure 26b), possibly owing to self-cleaning effects of the loose gravel, which has previously been reported in a study by Sternecker et al. (2013b) analysing the effects of substrate restoration on spawning grounds of brown trout. Freeze-cores taken another 20 days later indicated that the substrate cleaning was still effective, as only marginal increases in fine sediment content (Mangfall, 5.1 ± 2.1%; Sims, 2.4 ± 0.3%) could be detected (Figure 26c, d).
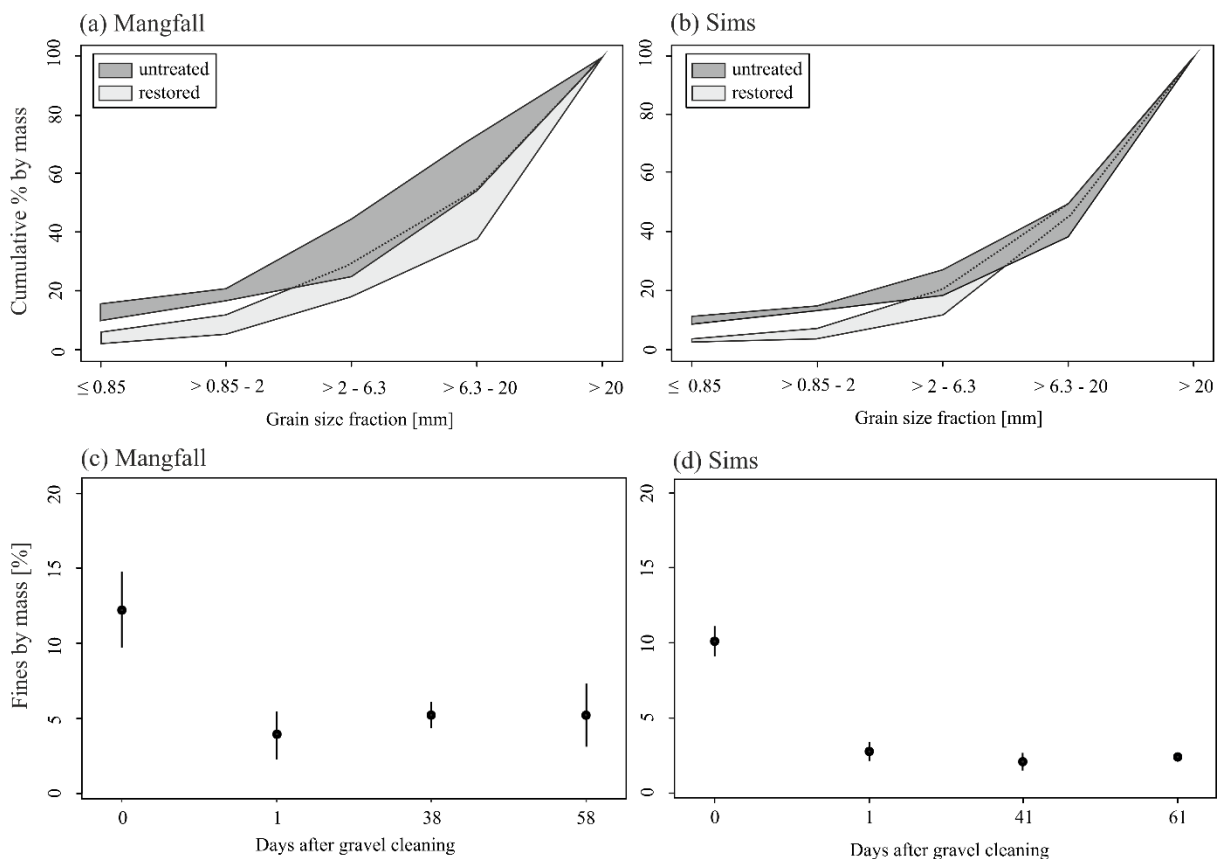
Figure 26: Direct effects of gravel cleaning on substrate composition in (a) the River Mangfall and (b) the River Sims (n = 3 each per river and treatment); grey shaded areas indicate the range between minimum and maximum values. Average (± standard deviation) development of fine sediment content (<0.85 mm) during the period of investigation in (c) the River Mangfall and (d) the River Sims (n = 3 per observation)

7.4.2 Spawning site use

In the River Mangfall, spawning of nase occurred on 6 and 8 April 2018. When spawning began, the water temperature was at 10.3°C and discharge at 1.5 m^3 s^{-1} (www.hnd-bayern.de; Water Authority Rosenheim, pers. comm., May 2018). In the River Sims, spawning occurred on 9 and 10 April 2018, at a water temperature of 11.3°C and a discharge of 1.47 m^3 s^{-1} (www.hnd-bayern.de).

Visual observations in the River Mangfall (conducted on 8 April 2018) recorded 281 spawning nase on the spawning ground, from which 148 individuals (53%) were counted within the restored site (density = 2.4 fish m^{-2}) and 133 within the untreated one (density = 2.1 fish m^{-2}). This observation was much more pronounced in the River Sims, where visual observations (conducted on 9 April 2018) counted 180 spawning nase, of which 160 (89%) were within the restored site (density = 3.3 fish m^{-2}), and only 20 within the untreated one (density = 0.4 fish m^{-2}). The observed visual effect on spawning site use was also evident in the number of deposited eggs in the spawning boxes, which was significantly higher at the restored sites compared with the untreated sites in both the River Mangfall (Kruskal–Wallis test: χ2 = 5.77; d.f. = 1; p < 0.05) and the River Sims (Kruskal–Wallis test: χ2 = 6.56; d.f. = 1; p < 0.05; Figure 27). In the River Mangfall, the number of eggs deposited at the restored site of the spawning ground was seven times higher (1,354 ± 1,394) compared with eggs deposited at the untreated site (262 ± 185). In the River Sims, this difference was even greater (restored 725 ± 811, untreated 38 ± 44). Combining the numbers of eggs deposited across sites and rivers, the mean number of eggs was twice as high in the first retrieval event (806 ± 1,249) compared with the second (383 ± 420), which occurred 6 days later; however, this difference was not statistically significant (Kruskal–Wallis test: χ2 = 0.003; d.f. = 1; p = 0.95).
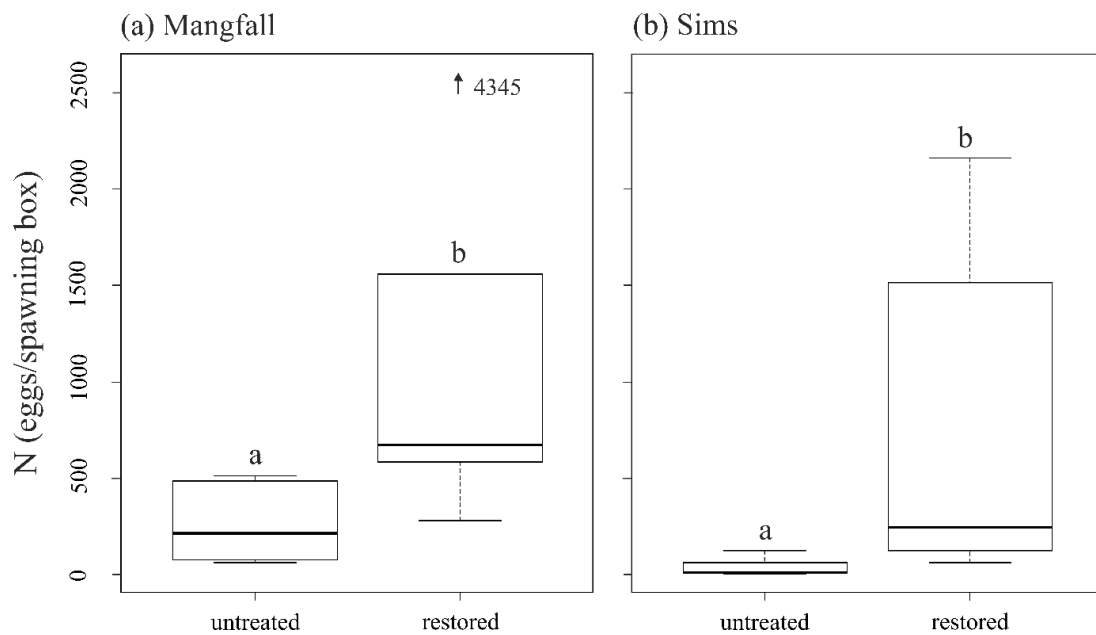
Figure 27: Distribution of eggs on the spawning grounds (n = 6 each). Box – 25% quantile, median, 75% quantile; whisker – minimum and maximum value. Extreme outlier is marked with a black arrow. Different letters above boxes indicate significant differences

7.4.3 Infiltration of eggs and larvae into the interstitial zone

Egg counts from freeze-core samples taken in the River Mangfall 6 days after spawning resulted in great differences between the restored (147 ± 121) and the untreated site (4 ± 1). The same was demonstrated for the River Sims (restored 28 ± 7, untreated 1 ± 1). In both rivers, eggs were detected down to a depth of 20 cm in the substrate, almost exclusively at the restored sites (Table 10). At the untreated site, only one egg in the River Mangfall was detected deeper than 10 cm in the substrate. Freeze-cores taken 26 days after spawning revealed no remaining eggs, but larvae down to 30 cm in the substrate (Table 11). Comparable with egg counts in the River Mangfall, the substrate layers of the restored site of this spawning ground contained most larvae (39 ± 31) whereas only a few (7 ± 4) were detected in substrate layers of the untreated site. In the River Sims, only four larvae were found, all in freeze-cores from the restored site

Table 10: Vertical distribution of eggs in freeze-cores (FC) taken 6 days after first spawning

Horizon [cm]	Mangfall						Sims					
	restored			untreated			restored			untreated		
0-10	91	17	213	7	2	3	23	21	14	0	0	3
>10-20	22	2	96	0	1	0	14	5	7	0	0	0
>20-30	0	0	0	0	0	0	0	0	0	0	0	0
FC Weight [g]	1,828	3,480	3,004	4,566	1,960	2,381	5,045	2,161	2,666	3,913	3,448	3,492

Table 11: Vertical distribution of larvae in freeze-cores (FC) taken 26 days after first spawning

Horizon [cm]	Mangfall						Sims					
	restored			untreated			restored			untreated		
0-10	6	19	48	7	10	1	3	0	0	0	0	0
>10-20	0	10	23	0	2	2	0	0	0	0	0	0
>20-30	0	2	9	0	0	0	0	0	1	0	0	0
FC Weight [g]	5,045	2,161	2,666	3,913	3,448	3,492	2,742	4,314	2,107	2,971	4,055	2,895

Drifting eggs were found downstream of all investigated sites, indicating that not all eggs were capable of infiltrating into the interstices of the stream bed or attaching to the substrate surface. Egg drift was highest during the days of spawning and declined consistently afterwards (Figure 28a, c). In the River Mangfall, the mean density (mean of the restored and untreated site) of drifting eggs was 0.61 ± 0.45 eggs m^{-3} on the first day of spawning (6 April 2018) and increased to the overall peak of 4.86 ± 1.54 eggs m^{-3} on the second day of spawning (8 April 2018; Figure 6a). The last eggs were found 189 dd after the first day of spawning. Egg drift densities from the restored site were significantly higher compared with the untreated site ($\chi 2(1) = 5.98$; $p < 0.05$). Mean drift density in the River Sims was 3.63 ± 3.55 eggs m^{-3} on the first day of spawning (9 April 2018). Comparable with the River Mangfall, the overall peak was reached on the second day of spawning (10 April 2018) with 9.57 ± 3.13 eggs m^{-3} (Figure 6c). At this time, the total numbers drifting downstream were estimated to be 50,000 eggs per hour. The last eggs were detected 159 dd after the first day of spawning (6 April 2018). No

statistical differences could be detected, although egg drift densities from the restored site in the River Sims exceeded those from the untreated site in every sample ($\chi2(1)$ = 3.37; p = 0.07).
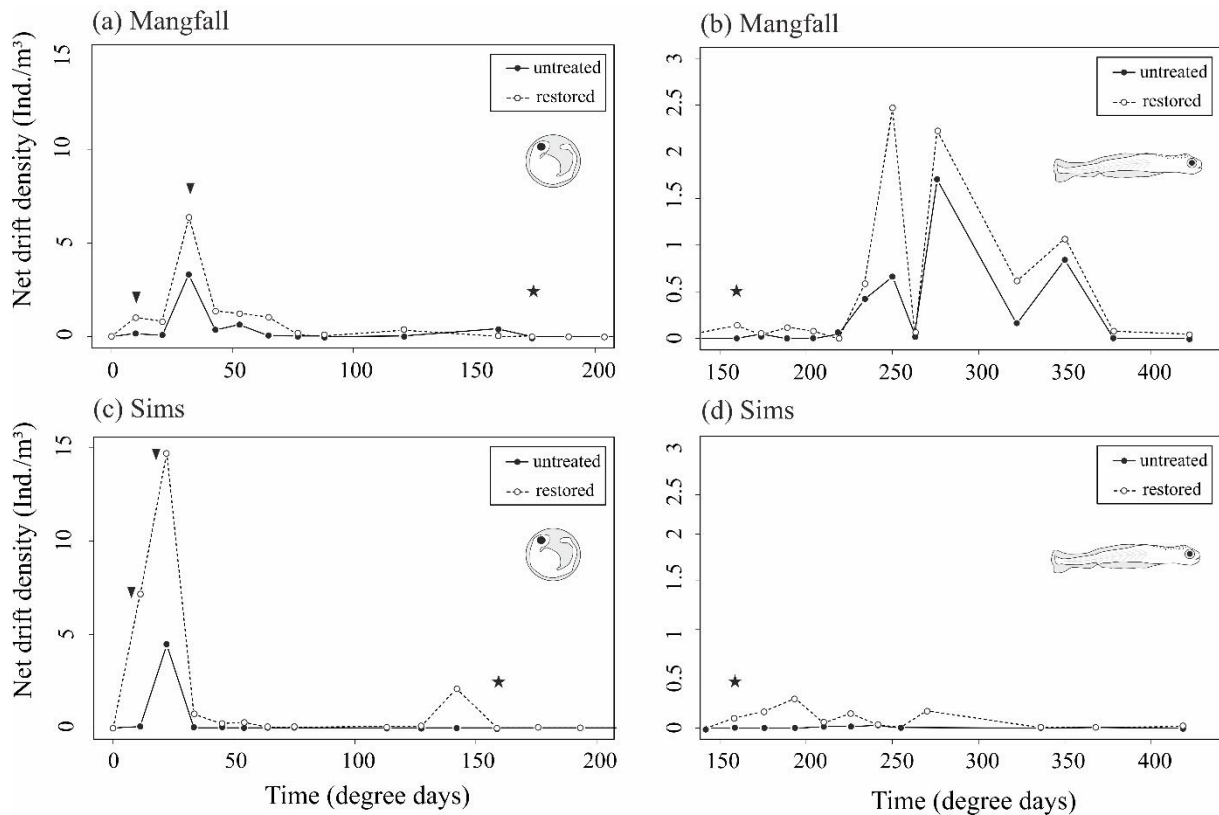
Figure 28: Drift densities of eggs (28a, c) and larvae (28b, d) from spawning grounds in the River Mangfall and the River Sims. Solid lines and filled dots indicate drift densities of organisms caught downstream of the untreated sites; dashed lines and circles indicate those caught downstream of the restored sites. Black triangles indicate spawning events, black stars show the catch of first larvae

7.4.4 Larval size at emergence

From a total of 3,139 larvae caught, 1,699 were morphologically intact and used for determination of total length. In the River Mangfall, 1,090 larvae (day 724; night 366) were measured from the treated site compared with 443 from the untreated site (day 311; night 132). From the treated site in the River Sims, 136 larvae were measured (day 110; night 26) compared with 30 from the untreated site (day 18; night 12). In all larvae measured, total length ranged between 7.2 and 15.0 mm. Larval length was significantly influenced by the factor daylight ($\chi2(1)$ = 16.65; p < 0.001), whereas treatment and river only showed a significant effect in the interaction term of these factors ($\chi2(1)$ = 4.03; p < 0.05). Only marginal differences in larval length were detected between the treated and untreated site, in the River

Mangfall (restored, 11.1 ± 1.4; untreated, 10.9 ± 1.3), as well as the River Sims (restored, 10.0 ± 1.3; untreated, 9.9 ± 1.0). At all sites, larval length increased over time (Figure 29) and was higher during the night (11.1 ± 1.2) compared with daylight sampling (10.7 ± 1.4). In addition, larval length was greater in the River Mangfall (11.0 ± 1.4) compared with the River Sims (9.9 ± 1.3)
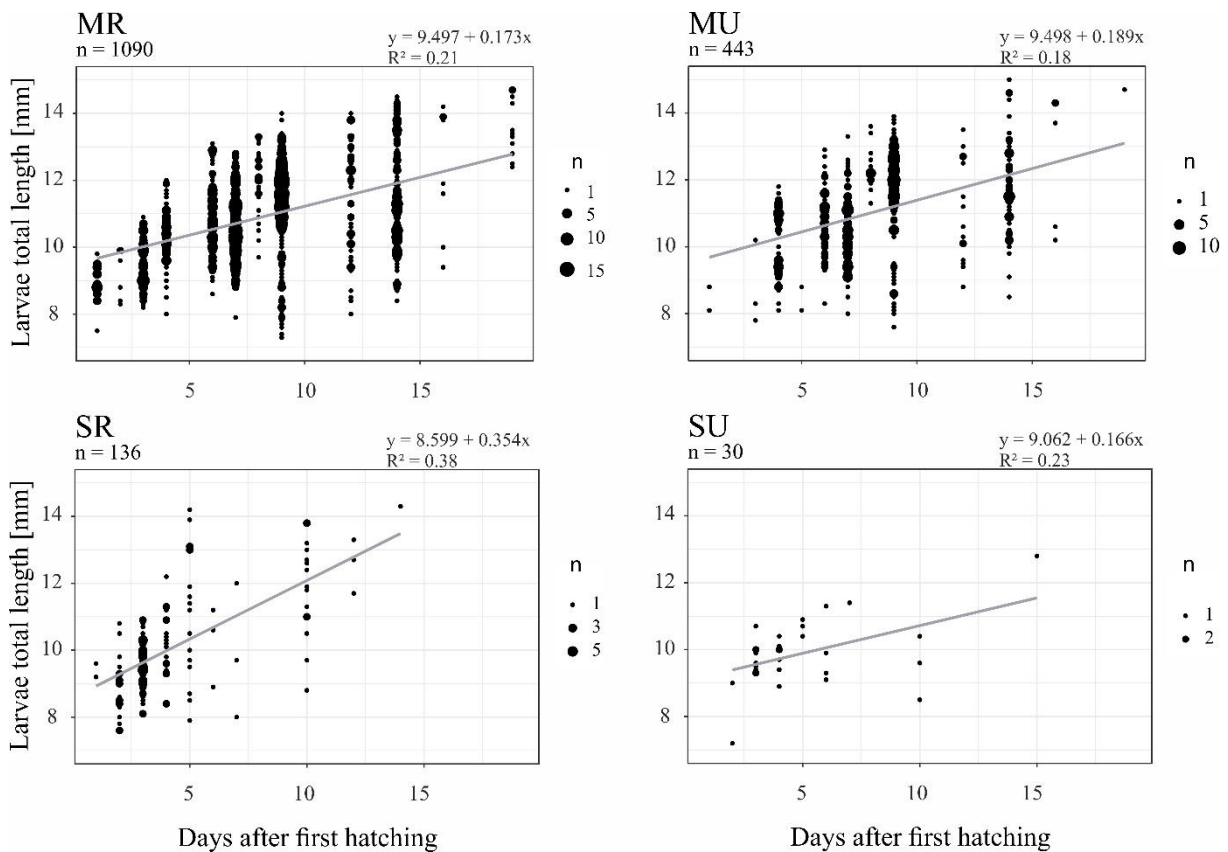
Figure 29: Weighted scatter plots showing the size of larvae caught while drifting downstream from the restored (MR) and untreated (MU) spawning ground in the River Mangfall and the restored (SR) and untreated (SU) spawning ground in the River Sims

7.5   Discussion

The results of this experimental restoration clearly indicate that cleaning of spawning gravel affects all crucial steps of the reproduction and recruitment success of nase as evident from increased use of spawning sites and numbers of eggs released as well as deeper infiltration of eggs and greater shelter of larvae in the interstitial zone, all resulting in greater larval recruitment.

7.5.1 Effects on nase spawning site use

In both rivers investigated, significantly higher numbers of eggs were found in restored parts of the spawning grounds, suggesting that the improved substrate quality, as indicated by the results of freeze-core samples, is a major factor influencing spawning habitat use. This interpretation is also supported by the number of spawning nase counted in the respective halves of spawning grounds, which was up to eight times higher in the restored parts. Although the number of nase counted in the spawning ground of the River Mangfall was only slightly higher in the restored site, the significantly higher numbers of eggs laid indicate that this site was preferred for egg release. The importance of substrate quality in spawning ground use has been demonstrated previously for salmonid species, e.g. with evidence of coho salmon (*Oncorhynchus kisutch* W.) spawning on substrates with certain gravel–pebble ratios (Mull & Wilzbach, 2007), as well as brook trout (*Salvelinus fontinalis* M.) preferring spawning substrate with reduced fine sediment content (Bernier-Bourgault & Magnan, 2002). Spawning sites of nase are commonly characterised by shallow, fast-flowing riffles with a high proportion of gravel and pebbles (Keckeis, 2001; Melcher & Schmutz, 2010), yet the results of this study indicate that other substrate-related parameters, such as colmation or bulk-density, might also influence spawning site acceptance at the microhabitat level. It is conceivable that males check for substrate quality before spawning events by breaking up the gravel with their tails, which has been described by Ahnelt and Keckeis (1994) as a pre-spawning preparation in nase. Zoogeomorphic effects of spawning activity have also been reported for another lithophilic cyprinid, the European barbel (Gutmann Roberts et al., 2020). This clearly demonstrates the value that assessing microhabitat use may have for future river management and restoration projects, as shown by Santos et al. (2018).

7.5.2 Effects on the infiltration of eggs and larvae in the interstitial zone

Higher numbers of eggs and larvae were also found in the interstitial zone of the restored sites in both rivers, indicating that loosened and cleaned spawning substrate provides important interstices for both developmental stages. This confirms recent findings from Duerregger et al. (2018) that post-spawning eggs seep down into the interstitial zone and post-hatching

larvae retreat to greater depths. The importance of a loose and porous interstitial for the early ontogeny of nase becomes evident in the reduced risk of predation and uncontrolled drift of eggs and larvae developing in sheltered interstices in the gravel bed. In contrast, these risks are elevated for eggs directly exposed on the gravel surface (Keckeis et al., 1996; Persat & Olivier, 1995). Spawning ground restoration by gravel cleaning clearly supports the egg and larval infiltration mechanism, as indicated by the higher number of eggs and larvae in deep interstitial layers of up to 20 and 30 cm, respectively, which were found almost exclusively in the restored halves of spawning grounds. As studies on European barbel and common minnow show that eggs of these species can also be found in substrate layers down to 20 cm (barbel: Pinder et al., 2009) and 30 cm, respectively (common minnow: Bless, 1992), it is likely that the benefits of gravel cleaning observed in this study might be transferable to other species with a similar ecology and incubation time, such as barbel, chub, dace and common minnow.

However, while gravel cleaning might be a good choice for species with a short incubation phase, this is still only a temporary solution (Mueller et al., 2014). In contrast, it is highly doubtful that single instream measures provide a sufficient conservation tool for aquatic biota that depend on a loose and well-oxygenated stream bed for a much longer period of time, such as freshwater pearl mussels (Denic & Geist, 2015). For these species sufficient habitat conditions can only by established when integrative catchment concepts, comprising management of land use and flow dynamics, are developed (Denic & Geist, 2015).

Generally, nase eggs are characterised by a cover of adhesive villi on the zona radiata externa (Patzner et al., 2006), supporting egg adhesive ability even in fast-flowing areas of spawning grounds. However, not all eggs are capable of remaining on spawning grounds, as high densities of suspended eggs drifting downstream were found during the days of spawning, exceeding those reported by Hofer and Kirchhofer (1996), in which the mean density of drifting eggs peaked at 3.17 eggs m^{-3}. Peaks in the drift of eggs were higher from the restored sites of spawning grounds, which can be explained by the significantly higher number of eggs laid on these sites.

In a study on egg populations of dace, another lithophilic cyprinid with a similar egg attachment mechanism (Petz-Glechner et al., 1998), the number of downstream drifting eggs directly linked to the initial egg population was estimated to be 2% in the wild (Mills, 1981).

Species-specific differences in the density and length of adhesive villi have already been reported (Riehl & Patzner, 1998); however, it remains unclear whether there are also intraspecific differences, as previously demonstrated by Keckeis et al. (2000), for egg size of nase, or effects of water chemistry on the attachment mechanism that might explain differences in the adhesiveness of eggs between the two rivers investigated. Moreover, it remains to be tested whether eggs drifting from spawning grounds can settle and develop elsewhere, or whether they are completely lost to the population. The latter seems likely in cases where streams transport high loads of fines, as observed by Nagel et al. (2020a), where even 10% fine sediment content in the incubation substrate (<0.85 mm) caused elevated mortality of nase eggs. However, drifting eggs can also contribute indirectly to the recruitment success by distracting predators from eggs developing in more favourable conditions on spawning grounds. In any case, our findings highlight the importance of a loose and porous interstitial on spawning grounds, as the chances of a successful development of eggs with a reduced adhesive ability is elevated if the stream bed provides sufficient porous space for egg infiltration.

7.5.3 Development success and timing of emergence

In both rivers, larval emergence was distinctly higher from the restored sites of spawning grounds, which can be linked to the preferred use of these sites by spawning nase. Subsequently, higher numbers of eggs laid develop in a greater interstitial space, which results in higher numbers of emerging larvae. It seems likely that the observed effects are not only a matter of a higher number of eggs laid, but also a result of more favourable conditions for the early ontogeny of nase, as the hatching rate of nase larvae increases with reduced fine sediment content in the incubation substrate (Nagel et al., 2020a). However, the variability in the development success between the two rivers was high, with a peak density of larvae emerging in the River Mangfall 12 times higher than in the River Sims. We assume that this could be related to several factors such as substrate composition or compaction, differences in water chemistry or biological causes such as a reduced egg adhesive ability of the Sims population, as indicated by the high densities of downstream drifting eggs and consequently lower numbers of eggs remaining on the spawning ground. As larval emergence in the River

Sims was almost exclusively observed at the restored site, it is possible that this population has fundamental problems in recruitment success under naturally occurring conditions that might threaten its survival. In the River Mangfall, emergence activity was detected from the record of the first larvae until the end of the investigation, but the peak of emergence was observed 155 dd after the first record of hatching. This suggests that post-hatching nase larvae use the interstitial zone as a sheltered habitat for further development and emerge several days after hatching, which is consistent with findings from a laboratory experiment, in which the time of hatching and emergence in nase larvae differed by up to 156 dd (Nagel et al., 2020a).

Generally, nase larvae are described as negatively phototactic (Peňáz, 1974). However, contrary to findings of Hofer and Kirchhofer (1996) in the wild and results from Persat and Olivier (1995) under experimental conditions, a shift to increasing drift activity of nase larvae during darkness in both rivers was not observed. Taking into account the high emergence success in the River Mangfall (derived by the high numbers of drifting larvae and findings of Duerregger et al., 2018), we assume that the main driver of dispersal in this spawning ground can be related to the reaction of larvae to population density effects after hatching (Lechner et al., 2016). The aggregation of eggs and larvae on spawning grounds can attract predators, such as chub – which were observed on spawning grounds after spawning of nase – waterfowl and macroinvertebrates (Keckeis et al., 1996; Persat & Olivier, 1995). A dispersal movement from spawning grounds can reduce these risks as well as the competition for space (Copp et al., 2002).

This interpretation is also supported by a study on brown trout (Daufresne et al., 2005) that demonstrated that downstream drift was reduced following displacement of 80% of the hatched fry. Moreover, the observed size difference in nase larvae drifting during daylight compared with those drifting during darkness suggests that daylight larval drift is mainly composed of small hatched embryos of eggs attached to the stream bed. Larvae are probably flushed away by the current immediately after hatching (passive drift entry) in contrast to larger individuals actively emerging from the substrate during the night (active drift entry). In addition, it remains unclear whether the observed size difference of larvae between the rivers investigated is a result of the time when larvae were caught in relation to the date of hatching or an inherited phenomenon caused by differing genetic constitution of the spawning

populations. The latter seems unlikely, as there are no migration barriers between the spawning grounds in the River Sims and the River Mangfall, and Wetjen et al. (2020) very recently detected only limited geographical differences in the genetic structure of nase poulations in Germany, where migration between populations was not interrupted. In contrast, a time-related effect seems more likely, as larval emergence in the River Sims stopped ~7 days earlier compared with the River Mangfall and a linear increase in growth of nase larvae following hatching is well described (Schludermann et al., 2009). Effects of greater larval sizes from the restored parts of spawning grounds were not observed in the present study; however, the effects of smaller larval sizes with increasing amounts of fines in the incubation substrate were found in studies under experimental conditions for nase (Nagel et al., 2020a) and for salmonids likewise (Sternecker & Geist, 2010).

7.6   Conservation implications

Identifying life stage specific deficiencies in habitat quality is one of the most crucial steps in developing sound conservation plans (Geist, 2011; Pander & Geist, 2013), yet knowledge of the autecological requirements of threatened European freshwater fish species is still not sufficient (Smialek et al., 2019). The results of this study reveal that spawning ground restoration for nase clearly supports the reproduction and recruitment success of this species, by reducing the amount of fines in the spawning substrates and thereby increasing the porosity of the stream bed. Consequently, the improved substrate quality results in a preferred use of the restored site, which is evident in the higher numbers of spawning nase and eggs laid on the spawning grounds. Considering subsequent development stages, the restoration-induced increase in stream-bed porosity leads to higher numbers of eggs and larvae infiltrating the interstitial zone, where larvae can successfully use the interstices as shelter, increasing emergence success and size at emergence. Other studies have observed only short-term effects of spawning substrate restoration and raise doubt concerning the effectiveness of this measure for species with a relatively long interstitial phase such as salmonids or freshwater pearl mussels (Mueller et al., 2014; Pander et al., 2015a). In contrast to those species, the predictability and synchrony of nase spawning runs in concert with a relatively short interstitial phase of less than 1 month, allowing a high accuracy in the timing

of spawning ground restoration and therefore greater restoration success. It is therefore important to consider spawning ground restoration as a quick, cheap and effective tool for the restoration and conservation management of nase and other species with a similar ecology and incubation time, such as barbel, chub, dace and common minnow. This holds especially true if streams lack internal dynamics and transport high fine-sediment loads. It is essential, however, to consider the spatial and temporal restriction of gravel cleaning effects as well as possible adverse consequences that may occur in downstream areas as a result of fine sediment wash-out when implementing this measure (Pander et al., 2015a). The extent of both depends on the individual geomorphology, flow dynamics and fine-sediment loads and is therefore highly river- and site-specific (Pander et al., 2015a). Consequently, long-term improvements for stream bed-dependent aquatic biota can only be achieved if all causes of fine-sediment clogging in the stream bed are addressed. This includes the reduction of fine-sediment loads from catchment-dependent land use as well as restricted gravel relocation caused by structural instream modifications (Geist & Hawkins, 2016). Rethinking common land-use practices, as well as re-establishing natural flow dynamics, is therefore crucial to support a self-sustaining process of reducing sediment input on the one hand and sediment mobilisation and relocation on the other.

8. Going with the flow: Spatio-temporal drift patterns of larval fish in a large alpine river

A similar version of this chapter was published in:

Candidate's contribution:

Study design and methodology was developed by CN with constant input from MM, JP and JG. CN conducted all fieldwork. Phenotypic identification and genetic species verification of ichthyoplankton was done by CN with constant input from BS, MM, JP and JG. Data preparation and statistical analysis, including all figures and tables, were conducted by CN. The original draft was written and finalised by CN. Revision and editing of the article were done by CN, MM, JP, BS, RK and JG.

8.1   Abstract

Fish larval drift is an essential step in the life cycle of riverine fish species as it determines dispersal and colonisation. Anthropogenic flow alterations and interruption of longitudinal and lateral connectivity by river damming and straightening can severely affect larval drift patterns. In this study, we characterised spatio-temporal drift patterns of fish larvae in the heavily regulated large alpine River Inn and within a constructed nature-like fish bypass.

Drift was investigated in the main reproduction period of the fish fauna in this river, ranging from mid-April to end of June. Diel patterns were assessed by samples taken during day, dusk, night, and dawn each day of sampling. To obtain robust species identification, we used DNA barcoding for genetic verification of phenotypically pre-sorted groups.

From a total of 1,069 fish larvae and 283 fish eggs caught, DNA barcoding revealed 16 species from five families, including several target species of conservation. We found strong evidence that several endangered species, such as *Chondrostoma nasus*, *Thymallus thymallus*, *Cottus gobio*, and *Aspius aspius* successfully reproduced in the bypass system. Genetic species verification showed a high level of homogeneity in most phenotypically pre-sorted groups.

Distinct seasonal patterns were observed, with the majority of fish species in the drift observed in mid-June. *Thymallus thymallus* and *Cottus gobio* dominated larval drift at the beginning of the observation followed by mainly unimodal abundance peaks of several Cyprinid species. Nocturnal drift prevailed in all species.

Our results on the spatial occurrence and abundances of fish larvae highlight the importance of bypass systems in heavily modified waterbodies to provide valuable spawning habitats and drift corridors around dams. Moreover, the distinct species-specific time patterns of larval drift represent a first basis to direct future discharge and river management plans in large alpine rivers towards a protection of the sensitive larval stages of specific target species of conservation. This includes bypass and turbine operation modes in the period of highest drift activity, as well as the construction of nature-like bypass systems and creation of spawning grounds therein.

8.2   Introduction

Anthropogenic alterations of stream morphology have resulted in a widespread loss in quantity, quality, and connectivity of habitats for riverine fish species (Aarts et al., 2004). This habitat loss ultimately evolved into a major cause for severe fish population declines, which is evident worldwide (e.g. Leidy & Moyle, 1998), but particularly in Alpine regions of Bavaria, Germany (Mueller et al., 2018). In this context, the early life stages of riverine fish have been identified as a particularly vulnerable life stage due to their high sensitivity to environmental disturbances (Schiemer et al., 2002). While knowledge of habitat requirements for spawning and embryonic development of target fish species for conservation is constantly growing (e.g. Nagel et al., 2020a; Sternecker & Geist, 2010 and references therein), there is less information on how limited connectivity of habitats in the early life stages may result in population effects.

After hatching and emergence, larvae drift with the current, which marks an essential step in the lifecycle of riverine fish (Lechner et al., 2016; Pavlov, 1994). Drift acts as an important dispersal mechanism from spawning grounds to suitable nurseries and in later ontogeny from nurseries to functional juvenile habitats (Pavlov, 1994). This mechanism is crucial, since aggregation of freshly hatched larvae at spawning grounds can lead to increased predation. Moreover, spawning habitats often do not provide the specific food sources riverine fish larvae need when shifting from endogenous to exogenous nutrition (Nunn et al., 2012; Reckendorfer et al., 2001). In addition, some riverine species such as common nase (*Chondrostoma nasus*, L.) require further habitat changes due to dietary shifts in their early ontogeny (Hofer & Kirchhofer, 1996; Reckendorfer et al., 2001). Moreover, drift is a major driver to extend population distribution ranges by (re-)colonising habitats, and it plays an important role in the gene flow among (sub-) populations (Lechner et al., 2016; Roberts et al., 2013). However, in regulated rivers, connectivity between habitats is often disrupted and larval drift impacted (Lechner et al., 2014b; Pavlov et al., 2020).

First, instream modifications such as damming interrupt longitudinal dispersal for downstream drifting larvae (Pavlov et al., 2020). In such cases and when no bypass system exists, downstream migration is only possible through the passage of spillways or hydropower turbines, where, in the latter, fish are exposed to the danger of blade strikes, shear forces, cavitation, and rapid pressure changes (Pracheil et al., 2016). To date, there is still little knowledge on how drifting fish eggs and larvae are affected when passing hydropower turbines and published results point in different directions, as effects heavily depend on the local conditions, the investigated impact and the species studied (Boys et al., 2016; Cada, 1990; Navarro et al., 2019). Second, river channelisation and bank stabilisation lead to a loss of lateral connectivity by altering shallow bank habitats, which provide important nurseries for the early life stages of riverine fish species (Jurajda, 1999; Pander et al., 2017). Drift studies conducted in large bank restoration sites at the Danube River revealed a changing species composition compared to the former heavily stabilised shore line (Ramler et al., 2016), particularly towards rheophilic cyprinids, when restoration featured shallow gravel banks (Meulenbroek et al., 2018a). Third, instream obstacles, such as weirs, reduce flow velocities in upstream areas, which in turn leads to a higher infiltration rate of fine grain sizes into the stream bed (Geist & Hawkins, 2016; Mueller et al., 2011). As a result, the vertical connectivity for hatched larvae of gravel spawning species is restricted, as fine sediment infiltration is

known to physically block emergence movements (Nagel et al., 2020a; Sternecker & Geist, 2010). Susceptibility to fine sediment infiltration during egg and larval development are evident in both the family of salmonids (Greig et al., 2005a; Kemp et al., 2011; Sternecker & Geist, 2010) and cyprinids (Bless, 1992; Duerregger et al., 2018; Nagel et al., 2020a). Consequently, the numerous river modifications firstly reduce the quantity of habitats for the early life stages, and the few remaining habitats reveal a reduced quality and connectivity.

Throughout Europe, efforts to restore the quality and connectivity of river systems are undertaken, targeting the objectives formulated in the Water Framework Directive (WFD; Council of the European Communities, 2000). The evidence of drifting early life stages allows validating the presence and functionality of spawning habitats and thus can be used to gain information on reproduction success of the ichthyofauna in a certain river section (Humphries & Lake, 2000). However, larval drift studies are a barely used tool to investigate river restoration success (but see Meulenbroek et al., 2018ab; Ramler et al., 2016).

Biological responses to river restoration are known to be species- and river-specific, which explains the insufficient success of measures that are not tailored to the species-specific needs of certain life stages (Pander & Geist, 2013). While the phenotypic identification of adult and juvenile fish is common standard, identification of fish larvae to species level often remains uncertain, even for experts (Ko et al., 2013). This holds especially true in situations with multiple morphologically similar fish species whose larvae co-occur. However, most studies in the past only relied on phenotypic approaches for larvae identification, even though some recent studies have also integrated genetic tools (Meulenbroek et al., 2018ab).

Although the body of knowledge on species-specific seasonal and diel drift patterns for fish larvae in lowland rivers is constantly growing (e.g. Johnson & McKenna, 2007; Jost et al., 2016; Oesmann, 2003; Reichard et al., 2004; Sonny et al., 2006; Zitek et al., 2004ab), large rivers within alpine catchments are to date distinctly underrepresented in the scientific literature. At the same time, knowledge on drift patterns in all existing river types is highly relevant, since spawning time and duration of egg incubation differ distinctly in fish species of temperate zones, mainly governed by water temperature-related effects, which ultimately cause a pronounced seasonal variation of drift patterns. Moreover, spatio-temporal information on drift patterns are even more important in regulated rivers as restoration measures and fish

protection facilities on hydropower plants, e.g. with regard to design and operational features of hydropower turbines, can be implemented accordingly.

Consequently, the main aim of this study was to investigate the drift of larval fish in a heavily regulated large alpine river including analysis of the taxonomic composition (verified to species level by DNA barcoding), temporal drift patterns as well as ontogenetic- and size structure of drifting larvae. The second aim was to assess spatial drift patterns between the main stem and a restored near-natural bypass channel, intended to provide additional spawning habitats for lithophilic species. Specifically, we hypothesised that the synchrony of drifting species in this large alpine river changes seasonally and that drifting eggs and larvae of all lithophilic species are found in higher densities within the bypass than within the main river owing from spawning activity of these species in the bypass.

8.3   Material and Methods

8.3.1 Study area

This study was conducted in the River Inn (Figure 30), a large alpine river ranked number 4 in terms of discharge of rivers in Germany. The Inn rises in the Engadin, Switzerland and flows over a stretch of 517 km, which mainly extends over Austria and Germany. Finally, it discharges at the city of Passau (Bavaria, Germany) with a mean annual flow (MQ) of 738 m³/s (hnd-bayern.de) into the Danube River (MQ = 689 m³/s, hnd-bayern.de). Bank stabilisation, restricting lateral connectivity, between the 17th and 19th centuries, and impoundment, restricting longitudinal connectivity, for the production of electricity in the 20th century changed the hydro-morphology and the resulting reduction of transport capacity for coarser sediment fractions of the formerly furcating River Inn dramatically. In addition, catchment land use change and the resulting elevated infiltration of fine sediment into gravel banks also restricts vertical connectivity by hampering the exchange between the hyporheic and the lotic zone. Because of its alpine catchment, the Inn is fed by meltwater from snow and glaciers during spring and summer, resulting in a fluctuating discharge, relatively low water temperatures and high loads of suspended sediments in the spawning season of the majority of its ichthyofauna (April–June; Table 12). Drift was investigated in the main river and additionally in a nature-like fish pass at the Gars am Inn hydropower station (48°15'N;

12°31'E). This system resembles a typical shallow and fast flowing side branch of the Inn, as it occurred manifold in the former braided course.

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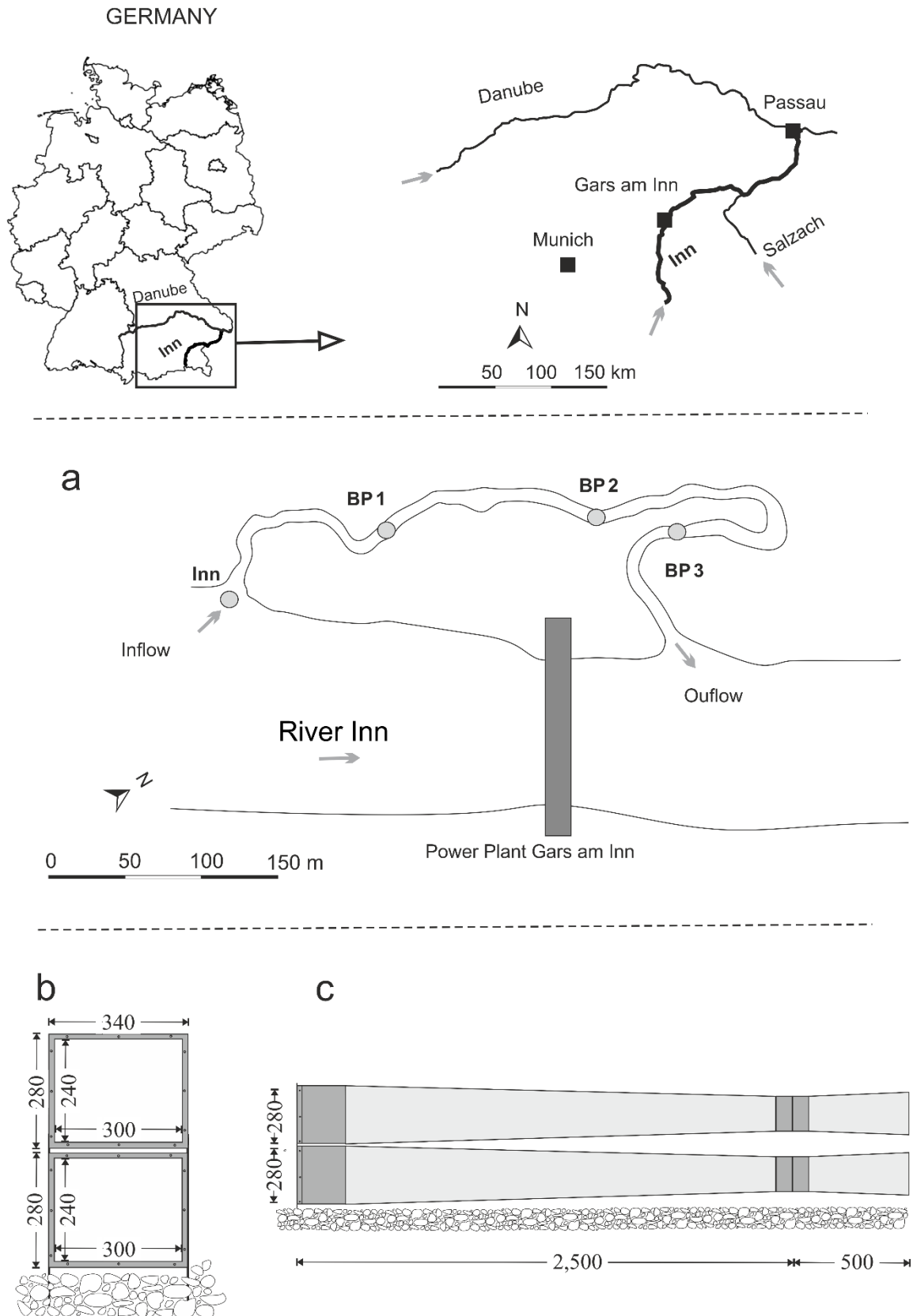
Figure 30: Location of the study area, positioning of sampling sites (a), front view (b) and side view (c) of the drift net setup. Dimensions in (b) and (c) are given in mm. Grey arrows indicate flow direction

Table 12: Abiotic variables measured during the period of investigation; values are given as means ± standard deviation. T = Temperature, O₂ = oxygen concentration, EC = electric conductance (related to 20°C)

Sampling dates	T [°C]	O$_2$ [mg L^{-1}]	EC [µS cm^{-1}]	pH	Turbidity [NTU]	Discharge River Inn [m³ s^{-1}]
April 18-21	8.2 ± 0.0	12.1 ± 0.1	350 ± 2	7.7 ± 0.1	7.5 ± 0.7	227 ± 2
April 24-27	9.5 ± 0.1	12.3 ± 0.4	357 ± 11	6.8 ± 0.1	7.0 ± 1.0	246 ± 36
May 02-05	10.4 ± 0.0	11.7 ± 0.5	357 ± 13	6.7 ± 0.0	12.2 ± 0.7	309 ± 6
May 08-11	10.4 ± 0.1	11.5 ± 0.1	322 ± 2	6.9 ± 0.0	24.0 ± 15.3	379 ± 30
May 15-18	10.4 ± 0.1	11.7 ± 0.4	357 ± 27	6.7 ± 0.9	12.2 ± 8.4	309 ± 3
May 22-25	13.6 ± 0.2	10.9 ± 0.4	278 ± 4	7.9 ± 0.1	25.9 ± 15.8	451 ± 53
May 29 - June 01	12.4 ± 0.4	10.8 ± 0.2	247 ± 33	7.1 ± 0.1	41.7 ± 32.8	400 ± 74
June 06-09	14.1 ± 0.1	10.8 ± 0.2	182 ± 1	7.2 ± 0.8	87.7 ± 44.8	564 ± 16
June 12-15	15.3 ± 0.1	10.2 ± 0.1	216 ± 20	7.2 ± 0.1	70.9 ± 27.0	498 ± 38
June 20-23	16.5 ± 0.5	10.4 ± 0.1	212 ± 8	7.9 ± 0.2	51.5 ± 4.1	466 ± 7
June 26-29	16.2 ± 0.6	9.8 ± 0.1	199 ± 2.1	7.5 ± 0.1	164.3 ± 54.0	490 ± 12

Abbreviations: EC, electric conductance (related to 20°C); O$_2$, oxygen concentration; T, temperature

8.3.2 Study design

8.3.2.1 Seasonal and diel patterns

To investigate seasonal patterns in the drift of larvae and young juvenile fish (for definitions see Table 13), sampling was conducted over a period of 11 weeks from 18 April to 28 June 2017 with samples taken at two non-consecutive days each week. To account for diel drift patterns, a standardised protocol was established comprising six sampling times each day of sampling. Based on this, nets were simultaneously set every c. 4 hr starting at 10 a.m. and ending at 6 a.m. the following day. This approach covered samples during dusk, day, night and dawn. Since the time of dusk and dawn changed during the period of investigation, these sampling times were adjusted accordingly. Other drift studies reported damage on trapped fish larvae, e.g. by the pressure of water or drifting debris (e.g. Penáz et al., 1992), which is likely to hamper the subsequent taxonomic identification of larvae. Therefore, to minimise larval damage for phenotypic pre-sorting, nets were only exposed for 30 min during each 4 hr sampling interval.

Table 13: Definition of the larval fish terminology used in this study

Term	Definition	According to
Free Embryo	Yolk sack still present	Pinder (2001)
Young larvae	Yolk sack absent but dorsal fin rays not yet visible	Pinder (2001)
Intermediate larvae	Evidence of fin rays within dorsal fin but not separated from the dorsal fin-fold posteriorly	Pinder (2001)
Older larvae	Dorsal fin completely separated from the fin-fold, but pelvic fin-fold still present	Pinder (2001)
Young Juvenile	No remaining fin-fold, all fins fully developed	Pinder (2001)
Ichthyoplankton	Fish eggs and larvae that are found drifting with the current	Moser & Watson (2006)

8.3.2.2 Spatial patterns

Larval drift was investigated at four sites: one in the main river in 3 m distance to the inlet of the bypass and 1 m distance from the shore and three sites along the flow course of the bypass to provide information on spawning activities within the fishway by spatially comparing drift densities of eggs and larvae (Figure 30a). Sampling sites were selected so that they were equally distributed along the course of the bypass, considering similar water depths (Table 14). To account for differences in the horizontal distribution of drifting eggs and larvae, two nets were placed on top of each other at each sampling interval, covering the whole water column at all sites. This resulted in an overall sample number of 4,224 during the whole period of investigation. Drift nets were constructed using rectangular aluminium frames for the mouth and tear-proof polyester (155 meshes/cm²) for the nets (Figure 30b, c; see also Nagel et al., 2020b).

Table 14: Flow velocity and water depth measured at the sampling sites. Values are given as means ± SD [range]

Site	Flow velocity [ms^{-1}]	Depth [m]
River Inn	0.12 ± 0.03 [0.05-0.20]	0.63 ± 0.03 [0.57-0.70]
BP 1	0.67 ± 0.14 [0.13-0.94]	0.58 ± 0.02 [0.55-0.62]
BP 2	0.57 ± 0.13 [0.20-0.80]	0.57 ± 0.02 [0.53-0.61]
BP 3	0.65 ± 0.11 [0.33-0.93]	0.59 ± 0.02 [0.56-0.63]

8.3.3 Sample processing and taxonomic identification

Subsequent to the collection, drift samples were immediately checked for fish larvae and eggs. Ichthyoplankton was anesthetised and killed with a 20-fold overdose (concentration according to Adam et al., 2013) of MS-222 (tricaine methanesulfonate) and subsequently preserved in 96% ethanol.

In the laboratory, taxonomic identification to family level, length measurement and determination of development stage was conducted using a stereo-microscope Olympus SZX10 (Olympus Deutschland GmbH) with a magnification of 6.3 and the cellSens-Software (OLYMPUS CORPORATION; www.olympus-lifescience.com). Total length of all morphologically intact larvae was measured to the nearest 1 mm. Determination of development stage (one

embryonic, three larval, and one juvenile stage) of all morphologically intact larvae was conducted following the key of Pinder (2001) (Table 13). To ease readability, all development stages are referred to as fish larvae in the following, unless specifically stated otherwise.

8.3.4 Species determination using DNA barcoding

Several studies describe the difficulties in determining fish larvae to species level without using genetic tools (e.g. Ko et al., 2013; Meulenbroek et al., 2018ab; Ramler et al., 2016), especially in the early larval stages and when species belong to the same genera. The same applies for fish eggs, which have even less characteristics for a clear taxonomic identification. Therefore, larvae caught in this study were pre-sorted into taxonomic families in a first step. Since the families Cottidae and Gasterosteidae encompass only one species in the River Inn (Cottidae = *Cottus gobio*; Gasterosteidae = *Gasterosteus acculeatus*), larvae from these families could be directly determined to species level. In a next step, larvae were further classified into groups according to similar phenotypic characteristics using established identification keys (Cyprinidae: Pinder, 2001; Spindler, 1988; Percidae: Ramler et al., 2014; Urho, 1996). Classification was based on the criteria development stage, shape, pigmentation, shape of head and mouth, and positioning of fins (if developed). To verify the accuracy of phenotypic classification, sub-samples of larvae (≥15%) of each identified group were subsequently used for DNA barcoding. Sub-samples were randomly selected over the whole period of occurrence of each phenotypically identified group and mostly encompassed samples from all sites where this group occurred. In case DNA barcoding proved the homogeneity of a group (i.e. all analysed individuals belonged to the same species), the species identity was assigned to all other individuals of this group. Larvae that were damaged morphologically in such a way that they could no longer be phenotypically grouped were classified as unidentifiable. DNA barcoding was based on a fragment of the cytochrome c oxidase subunit I (COI) gene, which is widely applied in fish species identification (Bingpeng et al., 2018). NucleoSpin® Tissue kits (MachereyNagel) were used for DNA extraction from fish eggs and larvae following the manufacturer's protocol. Amplification of the COI fragment (c. 600 bp) was performed using the fish barcoding protocol with the primer C_FishF1t1 and C_FishR1t1 (Ivanova et al., 2007). Subsequently, the polymerase chain reaction products were cleaned (NucleoSpin®Gel and PCR Clean-up Kit, Macherey-Nagel) and sequenced (Genewiz). For species identification of eggs and larvae, the sequences were included in a query search

using GenBank's BLAST (Basic Local Alignment Search Tool; Genbank, www.ncbi.nlm.nih.gov/blast).

8.3.5 Juvenile and adult fish sampling

One month before and 1 month after drift sampling, electrofishing was conducted to characterise the juvenile and adult fish fauna in the study area following the approach of Pander and Geist (2010). Six stretches each of 30 m length in the fluvial part of the bypass and five connected stagnant ponds (44–130 m2) were fished wading with a hand-held anode using an 8-kW electrofishing device (Grassl). Caught fish were determined to species level, measured to the nearest 1 cm and subsequently carefully released. Fish sampling was conducted under permit no. 31–7562 (LRA FS to the Aquatic Systems Biology Unit of TUM, valid to 15 February 2022) following standard sampling procedures to catch and handle fish in accordance with national and European guidelines.

8.3.6 Abiotic variables

To calculate densities of drifting eggs and larvae, current velocity (m s^{-1}) was measured once each day of sampling at six points in front of each net opening, three at the upper and three at the lower end, using an electromagnetic water flow meter (Ott MF pro, Ott, Table 14). To further characterise abiotic conditions during the period of investigation, a hand-held Multi 3,430 device (WTW) was used to measure O2 (mg L^{-1}), temperature (°C), electric conductance (µS cm^{-1}, related to 20°C), and pH once each day of sampling between 3 and 5 p.m.

Turbidity (nephelometric turbidity unit, NTU) was measured accordingly with three 20 ml subsamples taken from running water on each site using a PhotoFlex Turb handheld field measurement unit (WTW). In addition, temperature loggers (Lascar Electronics Ltd; www.lascarelectronics.com) were used to hourly measure the water temperature during the whole period of sampling. Data on the discharge of the River Inn at this site were provided by the power plant operator (Verbund Innkraftwerke GmbH).

8.3.7 Data analysis

Prior to further analysis, drift densities expressed as number of eggs or larvae caught in 1,000 m³ of filtered water were calculated separately for each drift sampling device by multiplying the mean flow velocity by the area of the net opening and the duration of exposure. To analyse differences in the seasonal and spatial community composition of drifting larvae, Bray–Curtis-similarities from species abundance data for each day and site based on drift densities were computed in PRIMERv7. Based on this Bray–Curtis resemblance matrix, non-metric multidimensional scaling was performed to visualise seasonal changes and spatial differences in the larval fish community. Spatial differences in the larval fish community were visualised by metric multidimensional scaling of Bootstrap averages based on Bray–Curtis Similarities. By applying analysis of similarities (ANOSIM), the same resemblance matrix was used to test for significant differences among sampling months and among sampling sites.

Species-specific distribution over time was visualised in estimated kernel density curves using the packages ggplot2 and plotly and the function stat_density_ridges from the package ggridges in R (R Core Team, 2017). Prior to the statistical significance tests of diel differences between sampled time periods and the horizontal distribution of drifting larvae and eggs in the water column, Shapiro–Wilk and Levene tests were applied to check for normal distribution and homogeneity of variances. Since none of the analysed data followed a normal distribution or homogeneity of variances, differences were tested with Kruskal–Wallis tests and post hoc Mann–Whitney U tests using R (R Core Team, 2017). Distribution of larvae length over time was visualised in marginal scatter plots using the packages ggExtra and ggplot2 in R (R Core Team, 2017). To detect single or multiple spawning events, we compared species-specific larval sizes during the period of investigation. Therefore, linear regression analyses were calculated in R (version 3.5.1, R Core Team, 2017), using the sampling date as predictor and larvae total length as response variable (see also Ramler et al., 2016). Significance levels were set to $p < 0.05$ for all statistical analysis.

To ensure a concise presentation of the results, analyses regarding seasonal, diel, spatial and size-specific drift patterns were conducted for the five most abundant Cyprinids (*Chondrostoma nasus*, *Squalius cephalus*, *Barbus barbus*, *Abramis brama*, *Aspius aspius*), the

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most abundant Cottid (*Cottus gobio*), Percid (*Perca fluviatilis*), and Salmonid species (*Thymallus thymallus*).

8.4   Results

Over a period of 11 weeks, covering 22 sampling days and 4,224 drift samples, a total of 1,069 fish larvae and 283 fish eggs were caught. Encompassing all larvae, the Cyprinidae family was most abundant (60.6%), followed by the Cottidae (21.3%), Salmonidae (6.2%; incl. Thymallinae [6.0%] and Coregoninae [0.2%]), Percidae (4.1%), and Gasterosteidae (2.7%). Due to morphological damage, 55 larvae (5.1%) could not be assigned to a family.

8.4.1 Species composition

Out of 1,069 fish larvae and 283 eggs initially caught, a subsample of 211 larvae (19.7%) and 33 eggs (11.7%) were barcoded, encompassing samples from every site and the whole period of investigation. DNA barcoding detected 16 species from five families. Most phenotypic classified groups revealed a high level of homogeneity of the morphometric pre-classification and encompassed only one species. The detection of multiple species only occurred in the group of freshly hatched larvae (free embryos) and in larvae that were assigned as unidentifiably owing from morphological damage. In addition, two specimens of *Leuciscus idus* (L.) were found in groups where, apart from these, only *C. nasus* were detected. Based on the results of DNA barcoding *C. nasus* (27.0%) was most abundant, followed by *C. gobio* (21.3%), *S. cephalus* (13.6%), *B. barbus* (9.4%), *A. brama* (L.) (7.9%) and *T. thymallus* (6.0%). All other species were represented in shares <5% (Table 15, Supporting information 4).

In comparison to the juvenile and adult fish fauna, 14 species could be exclusively detected via electrofishing whereas four taxa (*Aspius aspius*, *Coregonus sp.*, *Leuciscus idus*, *Sander lucioperca*) could only be detected via drift sampling (Supporting information 4)

Table 15: Species distribution in scientific and common names of all larvae verified by DNA barcoding (barcoded) and all larvae that could be assigned to species level (total), their conservation status and affiliation to ecological guilds

| Species | | N | N | Conservation status | | | Reproduction | Flow |
Scientific name	Common name	(verified)	(total)	EU-FFH directive	Red List Germany	Red List Bavaria	guild	preference
Abramis brama	Common bream	14	84				Phyto/lithophilic	Indifferent
Alburnus alburnus	Bleak	9	9				Phyto/lithophilic	Indifferent
Aspius aspius	Asp	16	16	II	3	3	Lithophilic	Indifferent
Barbus barbus	European barbel	19	100	V	2	3	Lithophilic	Rheophilic
Chondrostoma nasus	Common nase	80	289		2	2	Lithophilic	Rheophilic
Coregonus spec.	Whitefish	2	2				-	-
Cottus gobio	Bullhead goby	9	227	II	2		Speleophilic	Rheophilic
Gasterosteus acculeatus	Three-spined stickleback	4	29				-	-
Gobio gobio	Gudgeon	1	1				Psammophilic	Rheophilic
Leuciscus idus	Ide	2	2		3	3	Lithophilic	Indifferent
Perca fluviatilis	Perch	8	42				Phyto/lithophilic	Indifferent
Pseudorasbora parva (*)	Stone moroko	1	1				-	-
Rutilus rutilus	Roach	1	1				Phyto/lithophilic	Indifferent
Sander lucioperca	Pike-perch	2	2				Phytophilic	Indifferent
Squalius cephalus	Chub	38	145				Lithophilic	Indifferent
Thymallus thymallus	European grayling	5	64	V	3	2	Lithophilic	Rheophilic
Σ		211	1,014					

Note: Conservation status is given according to Annex II or V of the Habitats Directive, Council of the European Communities (1992), Red List (RL) Bavaria and Germany (Bohl et al., 2003). Categories: 2 = highly endangered, 3 = endangered. The affiliation to reproduction guilds is based on the classification of Balon (1975), flow preferences on Zauner and Eberstaller (1999)

8.4.2 Seasonal patterns

When drift sampling started on 18 April, drift density was lowest (2.9 Ind./1,000m³) and only two species could be detected in the larval (*T. thymallus*, *C. gobio*) and one in the egg stage (*C. nasus*), from which *T. thymallus* was most abundant. Two days later the first embryos of *A. aspius* and *C. nasus* were caught. During April and May, species diversity remained low (3–6 detected species per sampling date); however, changes in species composition were noticed. Subsequently, highest species (10)—and drift density (42.5 Ind./1,000m³) were observed on 12 June. Over the period of investigation, species composition changed significantly (ANOSIM: Global R = 0.46, p < 0.001; Figure 31) and clear seasonal patterns could be detected (Figure 32). The change in species composition was most profound between April and June (ANOSIM: R = 0.75, p < 0.001), but also evident between May and June (ANOSIM: R = 0.44, p < 0.001) and April and May (ANOSIM: R = 0.25, p < 0.001). At the beginning of the observation during the last 2 weeks of April, *T. thymallus* was found to be the most abundant species, followed by *C. gobio* in the first days of May. Subsequently, *C. nasus* dominated larval drift until the end of May. In June, predominantly Cyprinids were found with abundance peaks of *B. barbus* (June 6th), *A. brama* (June 12th) and *S. cephalus* (20 and 28). While some species (e.g. *A. aspius*, *T. thymallus*, and *A. brama*) were found to drift only in a very limited period of time, *C. nasus* and *C. gobio* were observed during the whole period of investigation (Figure 32).
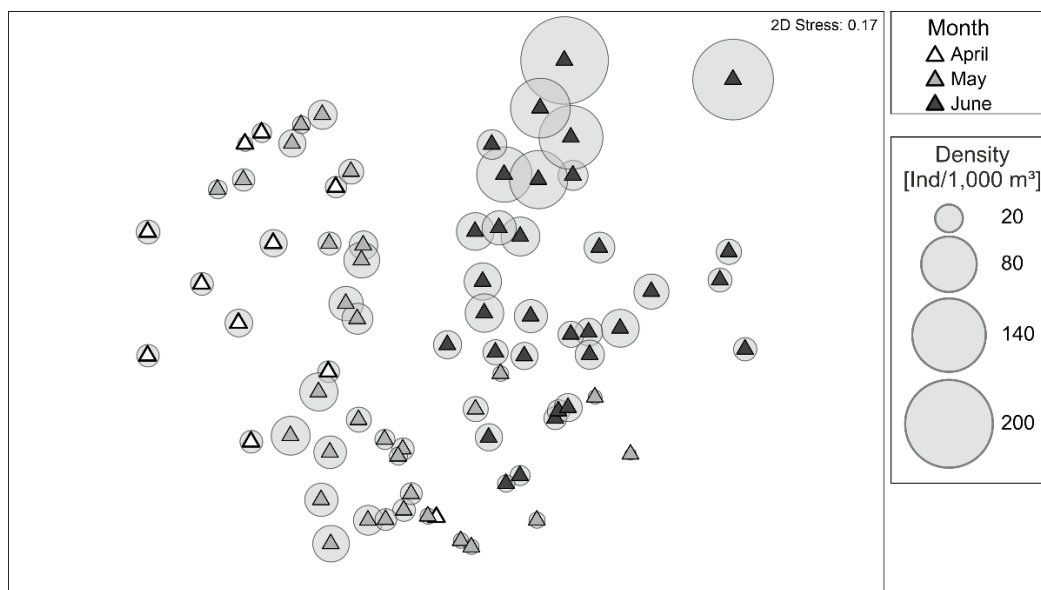
Figure 31: Non-metric multidimensional scaling of the seasonal distribution of the larval fish community composition based on drift densities. The distance between the symbols (summed up daily catch for each site) corresponds to the similarity in species community composition (small distance = large similarity). Drift densities are displayed as bubbles
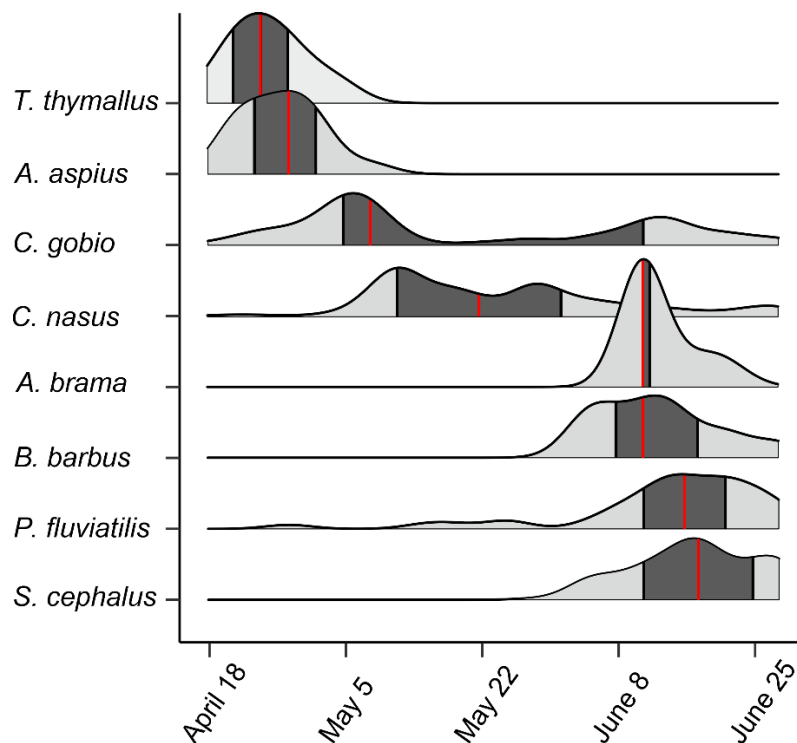
Figure 32: Density curves showing the estimated kernel density of larval drift for selected species. Quantiles (25%, 75%) are marked in dark grey, median is shown in red

Eggs of *C. nasus* were detected for a period of >3 weeks, from the beginning of the observation (18 April) until 10 May. Eggs of *S. cephalus* were caught for an even longer period, from 24 May until the termination of field sampling on 28 June. In contrast, eggs of *A. brama* were caught exclusively on 10 May.

8.4.3 Diel patterns

Considering all larvae caught, a clear peak in nocturnal drift was observed with the majority of larvae (36.0%) drifting during dusk (c. 10 p.m.) followed by those drifting at night (2 a.m.: 19.8%). Subsequently, a lower number of larvae was observed drifting during dawn (c. 6 a.m.: 11.7%) and daylight (10 a.m.: 9.6%, 2 p.m.: 10.1%, 6 p.m.: 12.8%). The shift to nocturnal drift activity was obvious in every species studied and *B. barbus*, *S. cephalus*, and *C. gobio* showed this pattern most strongly (Figure 33). In contrast, a less pronounced change in the diel abundances could be demonstrated for *C. nasus* and *T. thymallus*. In addition, the latter was the only species showing the highest drift abundance during 2 a.m. (Figure 33). A similar diel

pattern was observed for drifting eggs, which were caught to the highest share (36.4%) during dusk (c. 10 p.m.), followed by sampling times at 10 a.m. (21.9%), night sampling at 2 a.m. (20.1%), and dusk sampling at c. 6 a.m. (14.1%). The lowest number of eggs was detected during daylight sampling at 2 p.m. (2.8%) and 6 p.m. (4.2%).
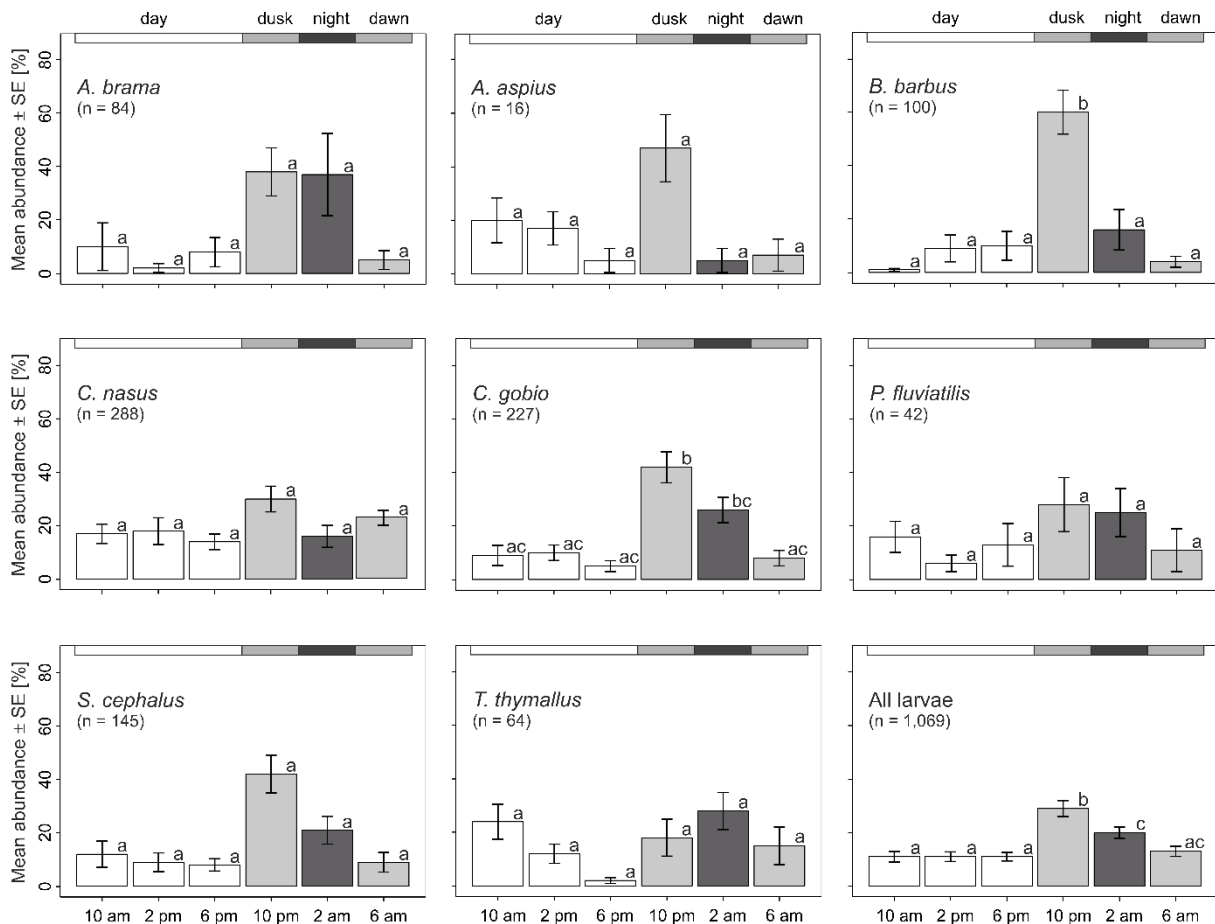
Figure 33: Species-specific mean diel abundance ± SE. Identical small letters above bars indicate statistically homogenous groups

8.4.4 Spatial patterns

Distinct changes in the spatial distribution patterns could be observed even within the c. 500 m long river stretch (ANOSIM: Global R = 0.14, p < 0.001; Figure 34). Larval drift in the main stem of the River Inn was mainly composed of *S. cephalus* (24.6%), *A. brama* (22.2%), *C. gobio* (22.2%), and *B. barbus* (15.1%) and changed significantly to the first site within the bypass (BP 1), located c. 100 m downstream of the division from the main stem (ANOSIM: R = 0.36, p < 0.001). There, *C. nasus* (28.3%) was most abundant, followed by *C. gobio* (28.1%) and *S. cephalus* (11.4%). At the subsequent BP 2 site, the same species were found in high

abundances (*C. nasus*: 28.6%, *C. gobio*: 14.5%, and *S. cephalus*: 14.9%), but additionally the presence of *T. thymallus* larvae was documented (11.3%; ANOSIM: R = 0.07, p < 0.05). At the most downstream site (BP 3) no significant changes in species distribution (*C. nasus*: 35.0%, *C. gobio*: 16.5%, *T. thymallus*: 12.7%, and *S. cephalus*: 10.4%) were detected compared to the BP 2 site (ANOSIM: R = −0.04, p = 0.99). *A. brama*, *B. barbus*, *C. gobio*, and *S. cephalus* were mainly caught in the nets investigating larval drift in the River Inn (Figure 35). In contrast to drift results of the main stem of the Inn, *C. nasus* and *P. fluviatilis* were found in much higher densities at sites within the bypass and two species, *A. aspius* and *T. thymallus*, were exclusively caught there (Figure 35). Eggs of almost all species were found at sites within the bypass, except for eggs of *A. brama* (exclusively caught on 10 May), which were only caught in the River Inn.
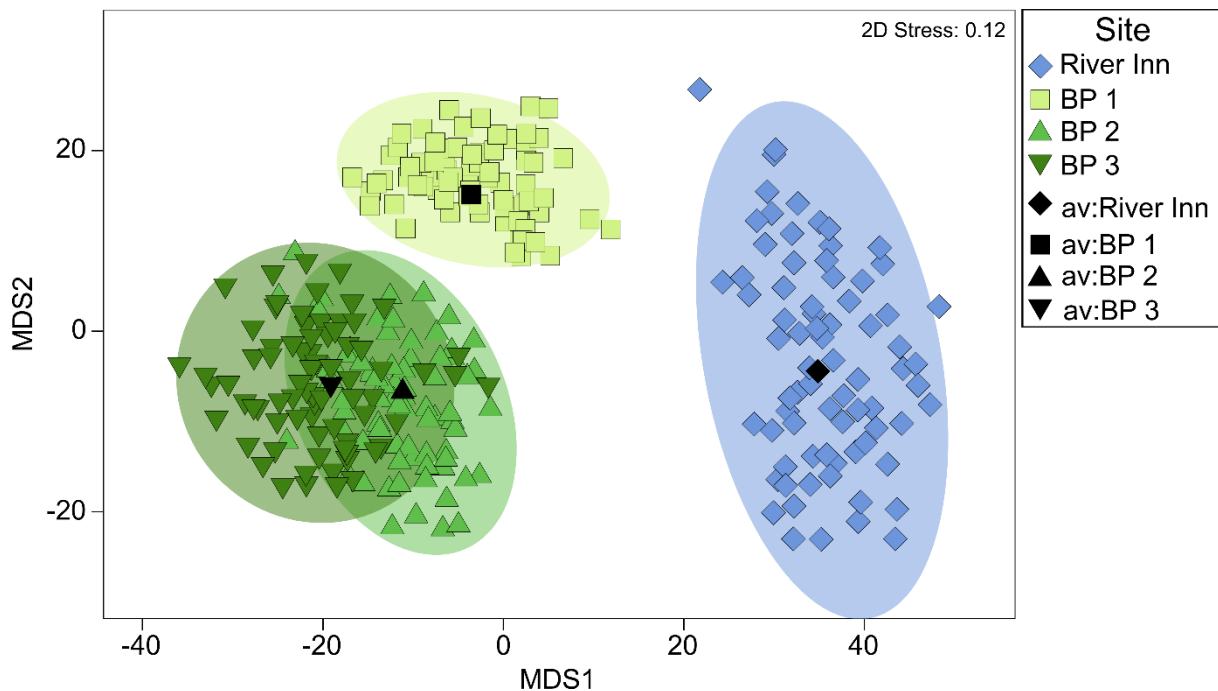
Figure 34: Metric multidimensional scaling (MDS) of bootstrap averages per site showing the spatial distribution patterns of the larval fish community composition based on drift densities. Bootstrap averages are based on pairwise Bray–Curtis similarities between each pair of sites calculated from the summed up daily species composition. Shaded ellipses represent more than 95% of all bootstrap average points of each site
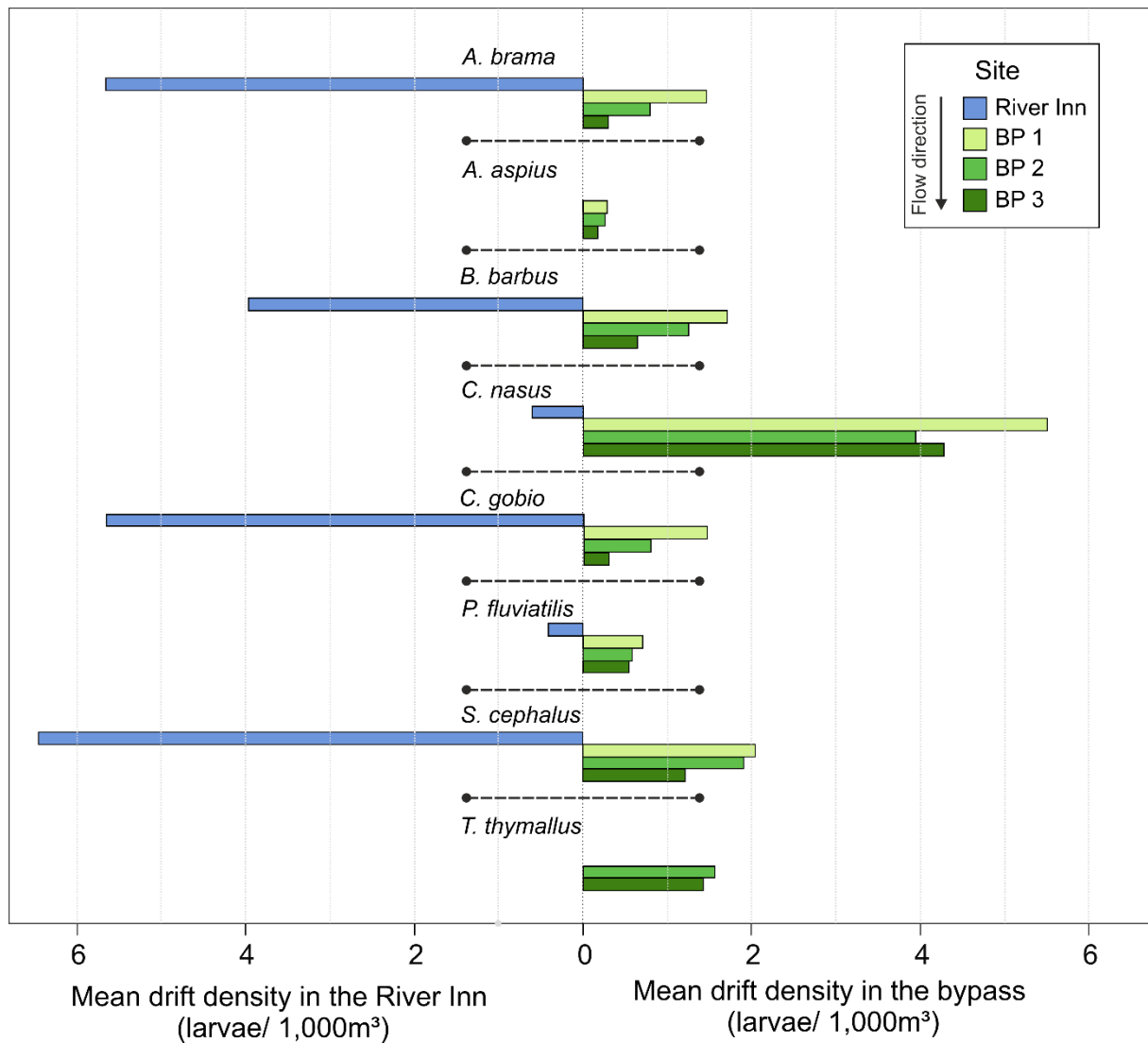
Figure 35: Species-specific mean drift densities at the four investigated sites Inn, BP 1, BP 2, and BP 3. Bars to the left refer to drift densities in the main stem of the Inn, bars to the right to drift densities within the bypass. Note that two species, Aspius aspius and Thymallus thymallus were exclusively found in the bypass

Average drift density of larvae (Ind./1,000m³) was higher in nets sampling the near surface water layer (17.9 ± 13.6) compared to nets sampling the near bottom water layer (13.6 ± 6.4), although this difference was not significant (Kruskal–Wallis test: χ^2 = 1.12; df = 1; p = 0.29). Drifting eggs were found in slightly higher densities (Ind./1,000m³) in the near bottom water layer (7.6 ± 16.9) compared to the near surface water layer (5.8 ± 7.6), but also no significant difference was revealed (Kruskal–Wallis test: χ^2 = 0.02; df = 1; p = 0.88).

8.4.5 Development stages and larval sizes

For all species, specific size-structures in the embryonic stage were observed and growth in the further ontogeny was species-specific (Table 16). In addition, most species also revealed an increase of larval length during the period of investigation (Figure 36), which was particularly evident in *C. gobio* (r^2 = 0.86; p < 0.001). However, this was not observed for *B. barbus* (r^2 = 0.02; p = 0.10) and *S. cephalus* (r^2 = 0.01; p = 0.28). Most larvae were caught during the young larvae stage (YL; 44.1%) and least larvae as free embryos (FE; 7.4%). All other development stages were represented in shares of 15.0%–20.0%. Some species, such as *C. nasus* and *C. gobio*, drifted in all developmental stages, while others were found drifting only in specific ones. *Thymallus thymallus* showed the highest share of free embryos (31.7%), while the majority of *P. fluviatilis* (87.8%) drifted as young juveniles (Table 16). Total length of all individuals caught ranged from 5 to 45 mm with both the smallest and the largest individuals being specimens of *P. fluviatilis*.

Table 16: Species and development stage specific measured larval total length L_T [mm] and abundances [%]

Species	Development Stage				
	FE	YL	IL	OL	YJ
A. brama					
L_T		10.8 ± 0.4	13.1 ± 0.7	13.9 ± 1.5	16.5 ± 0.5
%		4.9	15.9	76.8	2.4
A. aspius					
L_T	8.0 ± 1.0	10.6 ± 0.6			
%	12.5	87.5			
B. barbus					
L_T			14.6 ± 0.6	15.3 ± 1.5	17.0 ± 0.0
%			56.0	43.0	1.0
C. nasus					
L_T	9.5 ± 2.2	13.3 ± 1.2	15.1 ± 1.3	18.4 ± 1.8	23.4 ± 4.4
%	1.4	72.7	10.5	4.9	10.5
C. gobio					
L_T	9.1 ± 1.5	10.4 ± 1.0	10.5 ± 0.7	11.9 ± 1.8	21.0 ± 3.8
%	20.3	20.7	12.8	40.1	6.2
P. fluviatilis					
L_T	5.0 ± 0.0	12.5 ± 2.5		17.0 ± 1.0	32.9 ± 7.2
%	2.4	4.9		4.9	87.8
S. cephalus					
L_T	7.5 ± 0.5	9.8 ± 0.8	11.0 ± 1.4	13.9 ± 1.7	17.0 ± 1.0
%	1.4	87.3	4.9	4.9	1.4
T. thymallus					
L_T	16.4 ± 1.5	17.0 ± 1.1	21.0 ± 0.0		
%	31.7	66.7	1.6		

Note: *Classification of development stages is based on Pinder (https://onlinelibrary.wiley.com/doi/10.1111/fwb.13790#fwb13790-bib-0066) and comprises five stages: FE, free embryo; YL, young larvae; IL, intermediate larvae; OL, older larvae; YJ, young juvenile. Values for total length are given as means ± SD*
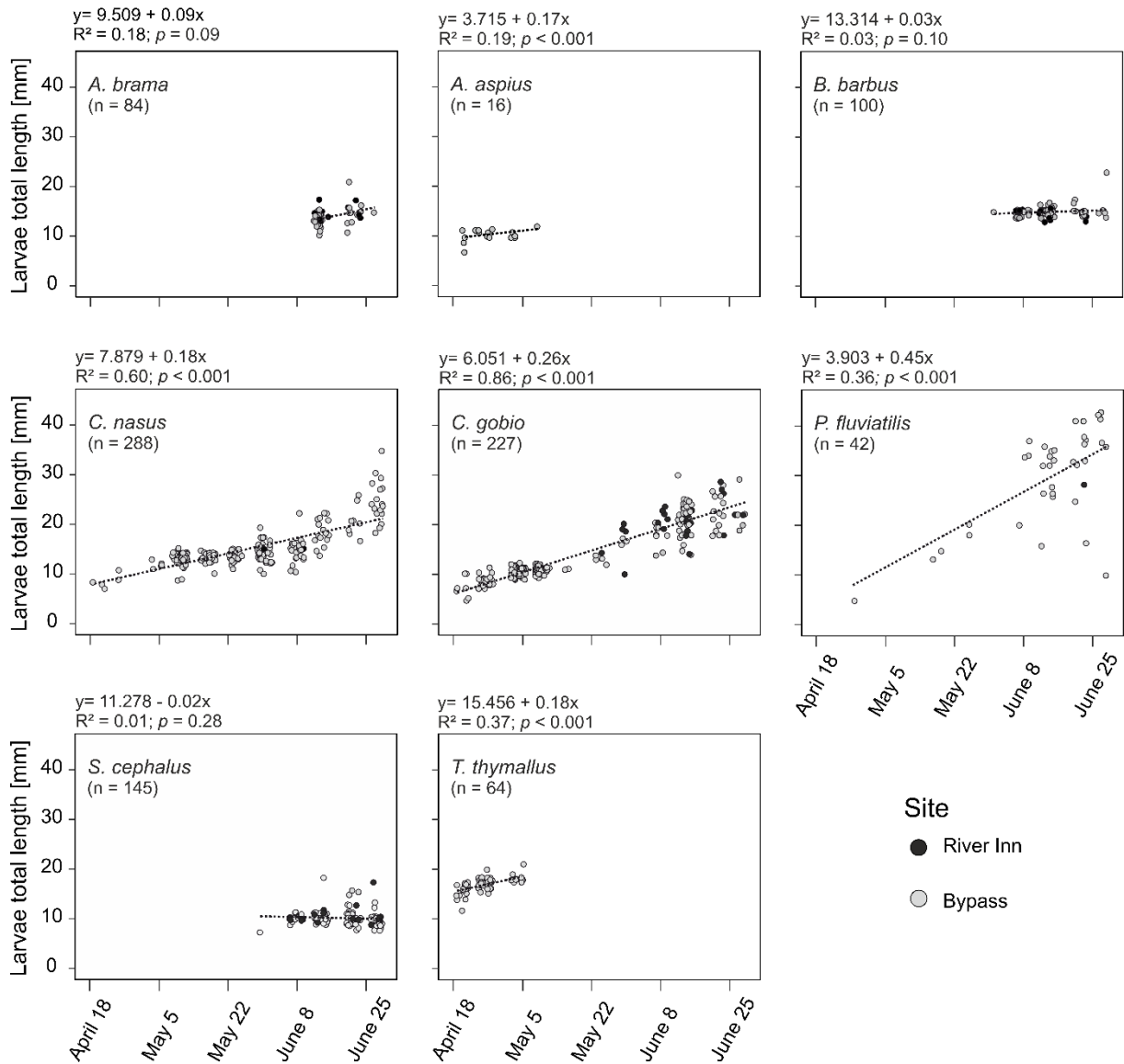
Figure 36: Scatterplots showing the distribution of larval total length over the period of investigation. Overlapping points are visualised using the function jitter in R. Black dots refer to larvae caught in the reference nets located in the River Inn, grey dots refer to larvae caught at the bypass sites

8.5   Discussion

With the unambiguous species identification obtained, we observed distinct species-specific seasonal and diel patterns in the drift of fish larvae in the large alpine River Inn. The results of this study also demonstrate the importance of including genetic tools in species identification of fish larvae, since not all morphologically pre-sorted groups revealed a 100% level of homogeneity. Moreover, the spatially resolved comparison of drift densities revealed profound differences in the species composition of the main stem and the nature-like bypass, which strongly indicates spawning activity in this side-branch.

These findings extend the existing knowledge of larval drift patterns in lowland rivers and can thereby support evidence-based restoration and hydropower management concepts in large alpine rivers.

8.5.1 Species composition

DNA barcoding detected 16 species, from which several, such as *C. nasus*, *B. barbus*, and *T. thymallu*s, have faced strong population declines in Bavaria (Mueller et al., 2018) and have therefore become target species of conservation. Moreover, *A. aspius*, *C. gobio*, and *T. thymallus*, together comprising 38.1% of all larvae caught, belong to species listed in Annex II or V of the Habitats Directive (Council of the European Communities, 1992) protecting them against uncontrolled removal (Annex V) and requiring Member States to designate special areas of conservation where these species occur (Annex II). The multiple proof of endangered and protected species in this study clearly demonstrates the importance of obtaining definite results in the taxonomic identification of fish larvae to the species level. This is of particular importance as protection measures, such as the designation of protected areas (Saunders et al., 2002), spawning ground improvements (Nagel et al., 2020b; Pander et al., 2015a; Taylor et al., 2019), and the design and operation of hydropower turbines (Boys et al., 2016; Cada, 1990; Geist, 2021) can be implemented according to the observed patterns of larval fish distribution.

The exclusive proof of larval stages for the taxa *A. aspius*, *Coregonus sp.*, *L. idus*, and *S. lucioperca*, which could not be detected as juveniles or adults via electrofishing, additionally

highlights the benefit of including larval drift studies in fish monitoring programs, especially if combined with genetic species identification.

Larval drift in the River Inn was mainly composed of lithophilic Cyprinids, *C. gobio*, and *T. thymallus*. Cyprinids were also most abundant in other drift studies from central European lowland rivers, yet rather ubiquitous species, such as *A. brama* (Jost et al., 2016; Reichard et al., 2004) or *Rutilus rutilus* (L.) (Sonny et al., 2006) dominated the species composition in these rivers. Drift studies in other Central European rivers invaded by gobies revealed that the species composition changed rapidly and fundamentally in the direction of goby species dominance (Jost et al., 2016; Meulenbroek et al., 2018a; Ramler et al., 2016; Zitek et al., 2004b). This shift holds particularly true for the invasive *Neogobius melanostumus* (P.), which originates from the Black Sea and is well known to cause ecological regime shifts by outcompeting other species that occupy the same ecological niche, such as the native *C. gobio*. However, non-native gobies have not yet been confirmed from the River Inn, although the invasion front of *N. melanostumus* in the Danube already extends far upstream of the confluence with the Inn (Brandner et al., 2013; Cerwenka et al., 2018).

With a peak density of 42.5 Ind./1,000 m³ larval density in the River Inn was considerably lower compared to peaks observed in other studies in lowland rivers of the Czech Republic (Reichard et al., 2004: 1,310 Ind./1,000 m³), Belgium (Sonny et al., 2006: >1,200 Ind./1,000 m), and Austria (Zitek et al., 2004a: >2,000 Ind./1,000m³). On the one hand, this can be linked to the alpine catchment of the River Inn, which results in relatively low water temperatures and high turbidity, ultimately limiting productivity of the water body. On the other hand, the River Inn suffers from multiple stressors, including heavy morphological alterations and erosion from agricultural land use, which additionally affects the recruitment success of its fish fauna (Bierschenk et al., 2019; Mueller et al., 2020).

8.5.2 Seasonal and diel patterns

A high interspecific variability in the seasonality of drift patterns was observed, which was also evident in the timing and duration of occurrence. Drift intensity is known to be species-specific and governed by a variability of factors such as discharge (e.g. Reichard & Jurajda, 2004) and temperature (e.g. Zitek et al., 2004b). These variables also influence spawning activity and

success, which is in turn directly linked to the intensity of larval drift (Lechner et al., 2016). Similar to findings from Zitek et al. (2004a) and Zitek et al. (2004b) who assessed drift patterns of 24 species in an artificially created side branch of the Danube River, drift activity in the Inn River increased with increasing temperatures. The same pattern was evident in our data, where drift activity (i.e. drift density of larvae) was directly linked to species diversity with more species being detected at high densities of drifting larvae. Rising water temperatures may explain this observation, as these are important stimuli for the induction of spawning migration and activity (e.g. Rakowitz et al., 2008) and additionally accelerate embryonic development (e.g. Kamler et al., 1998). As a result, hatching and drift of larvae from various species occurs synchronously.

On the first day of the investigation in mid-April, three rheophilic target species of conservation (*T. thymallus*, *C. gobio*, and *C. nasus*) could already be detected, contrasting to findings in lowland rivers, where larval drift was observed later in the year from May to July (e.g., Ramler et al., 2016; Reichard et al., 2004; Sonny et al., 2006; but see Meulenbroek et al., 2018a). This clearly indicates that monitoring of larval drift in rivers with an alpine catchment should start earlier in the year to also encompass late March and April.

Chondrostoma nasus and *C. gobio* were present during the whole period of the investigation, but peaks in the beginning of May can be attributed to a dispersal drift from spawning grounds, as confirmed by Nagel et al. (2020b) for *C. nasus*. Apparently, *C. nasus* directly spawned in the fishway since only two larvae were found coming from the River Inn and additionally eggs were detected multiple times in drift nets located within the bypass system. In contrast to *C. nasus*, which spawn their sticky eggs in large schools directly at the substrate surface and subsequently abandon spawning grounds (Melcher & Schmutz, 2010; Nagel et al., 2020b), female *C. gobio* stick their eggs underneath large stones where they are fertilised by a single male, which subsequently guards the eggs (Bisazza & Marconato, 1988). Our results indicate that, similar to *C. nasus* larvae, larvae of *C. gobio* enter the drift a few days after hatching following a dispersal movement from spawning sites, as evident from the multiple detection of free embryos and young larval stages in the first days of the investigation. This behaviour is most likely to follow a strategy to avoid density or filial cannibalism, since the latter has been described for egg guarding males preying on eggs of their own broodstock (Marconato et al., 1993). However, accidental drift entries cannot be fully excluded.

Predation avoidance in concert with a loss of visual orientation is also one of the most commonly used interpretations to explain the nocturnal shift in diel drift patterns of fish larvae (e.g. Copp et al., 2002; Gustafson-Marjanen & Dowse, 1983; Harvey, 1991). Contrasting to comparable studies in lowland streams, where the day–night ratio varied up to 1:190 (Reichard et al., 2004), the nocturnal shift in this study was not that distinctive as in lowland streams. High turbidity values measured in the period with highest drift activity in June may have strongly impeded the visual orientation and thereby drift activity of larvae, as effects of water transparency are described to reduce the nocturnal drift pattern (Oesmann, 2003; Pavlov et al., 2020; Reichard et al., 2001). However, we observed the highest drift activity during dusk in almost every species, which is in line with findings from other studies (e.g. Sonny et al., 2006) that describe a pronounced drift activity in the first hour after sunset.

Only *T. thymallus* showed a more balanced diel drift activity during day and night, which peaked at night sampling. In accordance with the results of this study, Van Leeuwen et al., (2017) demonstrated that drift of *T. thymallus* larvae in large rivers in Norway was highest during night. The pattern of downstream displacement of *T. thymallus* under experimental conditions is described as a two-step process in which emergence of *T. thymallus* larvae mainly occur during dusk followed by a resting phase in the first strata of the gravel bed until drift entry peaks during nightfall (Bardonnet & Gaudin, 1990). As Bardonnet & Gaudin (1990) conducted this study with very low flow velocities, it is likely that not all post-emergent *T. thymallus* in the wild are capable of resisting flow velocities occurring at spawning sites and could therefore be accidently displaced downstream throughout the day as well.

Comparable to larval drift, egg drift was also observed to occur mainly during dusk sampling. Since Nagel et al. (2020b) demonstrated that elevated egg drift downstream of *C. nasus* spawning grounds can be taken as a proxy for immediate spawning activity, it is highly likely that *C. nasus* and *S. cephalus*, which used the bypass for reproduction, mainly spawned during low-light conditions. This interpretation is also supported by findings of Šmejkal et al., (2018) who revealed a nocturnal spawning behaviour in the lithophilic cyprinid *A. aspius*, which was explained by a strategy to avoid egg predation of diurnal predators.

8.5.3 Spatial distribution and size

The pronounced spatial variability in species composition observed in this study indicates spawning activity differences between the investigated sites, as the presence of drifting larvae provides evidence of successful reproduction within a certain river stretch (Humphries & Lake, 2000). Spatial comparisons of drifting larval densities have therefore also been used to measure spawning success in large bank restoration sites (Ramler et al., 2016), nature-like bypass systems (Meulenbroek et al., 2018b), or spawning grounds (Nagel et al., 2020b). However, it has to be kept in mind that not all larvae drifting along the shoreline of the main stem of the Inn were necessarily caught in the drift nets placed there, since for technical reasons, such as river width and water depth, it was not possible to sample the whole cross-section of the bypass channel inlet. Therefore, it is highly likely that some larvae coming from the River Inn were also caught in drift traps located further downstream in the bypass. Our results indicate that this was the case for *A. brama* and *B. barbus*, as drift densities for these species constantly declined from upstream to downstream sites.

In contrast, larvae of *T. thymallus* and *A. aspius* were exclusively caught in the bypass, clearly indicating spawning activity of these species within this system. The same was true for all eggs of *C. nasus* and *S. cephalus*, providing strong evidence that these species also used the bypass for reproduction. These findings are in line with Pander et al., (2013) who demonstrated that nature-like fish passes can provide substitute habitats for rheophilic species, as well as Meulenbroek et al. (2018b) who revealed spawning of *C. nasus* and *B. barbus* in a nature-like fish pass of the Danube River. All the species we could prove to have used the bypass system for reproduction are classified as lithophilic (Balon, 1975) and require shallow gravel banks with medium to rapid current for spawning (*C. nasus*: Duerregger et al., 2018; Keckeis, 2001; Melcher & Schmutz, 2010; Nagel et al., 2020b; *A. aspius*: Horký & Slavík, 2017; *T. thymallus*: Gönczi, 1989; *S. cephalus*: Fredrich et al., 2003). Shallow gravel banks with high currents are among the most vulnerable riverine habitats as river damming and straightening decrease flow velocities while increasing water depths and fine sediment deposition (Geist & Hawkins, 2016; Mueller et al., 2011). Anthropogenic river regulation therefore often leads to a loss of these habitats. Our results demonstrate that nature-like fish passes resemble shallow fast-flowing site branches of the formerly furcating River Inn (see Pander et al., 2021) and thereby provide habitats that are degraded and partly lost in the nowadays heavily channelised main

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stem. This habitat function highlights the importance of near nature-like fish passes not only as migratory paths, but also as additional spawning grounds, which should be considered in future restoration efforts.

Additionally, we found strong evidence that the endangered *C. gobio* reproduces in the bypass as this species was found exclusively in the bypass during the first weeks of the investigation and additionally in young development stages. Only from 15 May onwards was this species also found drifting in main River Inn. Specimens of *C. gobio* hide under stones during the day (Knaepkens et al., 2002) and use them for reproduction by sticking their eggs on the bottom side, classifying them as speleophilic (Balon, 1975). In the bypass system, parts of the river banks are artificially stabilised with large stones, apparently also providing spawning habitats for this protected species.

The clear increase in larval size with the period of investigation in *C. gobio* further indicates a short spawning period for this species in the River Inn, probably owing from the low water temperatures of this river. This is consistent with findings from Fox (1978), who revealed single spawning behaviour in *C. gobio* originating from a less productive upland stream in contrast to multiple spawning events of populations in a more productive lowland stream.

A linear size increase of 0+ fish with ongoing larval development is described for several species (Limburg, 1996; Otterå, 1993; Schludermann et al., 2009). However, in our study we could not observe a significant relationship of larval size and time for every species studied. This was particularly evident in *S. cephalus* and *B. barbus* as well as in the first weeks of the investigation for *C. nasus*. These observations, in conjunction with the multiple proofs of drifting eggs of *C. nasus* (for >3 weeks) and *S. cephalus* (for 5 weeks) can probably be taken as an indicator for multiple spawning events of these species as previously observed by other authors (Gutmann Roberts & Britton, 2020; Peňáz, 1996; Poncin, 1989).

8.6 Conclusions

The results of this research demonstrate that fish larvae drift in a regulated large alpine river follows distinct seasonal and diel patterns, which represents a vital basis to direct future discharge and river management plans in large alpine rivers towards a protection of the sensitive larval stages of specific target species of conservation such as *C. nasus* and *T. thymallus*. In particular, information on seasonal drift patterns can be used to increase discharge of bypass systems in the period of highest drift activity, to strengthen the path leading larvae around instream obstacles. Moreover, information on diel drift activities might be useful to adjust turbine operation modes in accordance. However, more research on the annual variation of drift patterns, the distribution of drift paths in large rivers as well as on larval injuries in the turbine and spillway passage is needed, as these are likely to show a high annual and site-specific variation. The results of this study also prove that nature-like fishways do not only function as dispersal paths for downstream drifting larvae-even though for a reduced set of species-but can also provide important spawning habitats for target species of conservation. Monitoring and management of nature-like fishways is therefore crucial in catchment management plans of regulated rivers, since these systems may play an important role in the maintenance of aquatic biodiversity. This holds especially true in heavily modified water bodies, where the spawning potential for rheophilic species is limited and the dispersal of fish eggs and larvae severely altered.

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Candidate's contribution:

JP, CN and JG designed the study and developed the methodology. CN, JP and Hannah Ingermann (HI) conducted all fieldwork related to the study. JP and CN equally contributed to data preparation and statistical analysis, including all figures and tables. The original draft was written and finalised by JP, CN and JG. Revision and editing of the article were done by JP, CN, HI and JG.

9.1   Abstract

Bank habitats provide important functions for riverine fish, yet have been heavily modified in the context of land use, technical flood protection measures and hydropower installation. Fish species requiring specific habitats for the completion of their life cycle have strongly declined and therefore become target species of river restoration measures. This study compared abiotic conditions and fish community composition of three bank habitat types in a large alpine river, comprising different degrees of alteration compared to the natural state (concrete profile, bank riprap and naturally restored riverbank). Significant differences in abiotic habitat characteristics such as bed material, water depth, turbidity, submerged vegetation, and temperature were detected between the three bank habitat types and

sampling seasons. These water level dependent structural changes had the strongest effect on fish community composition as detected by distance-based linear modelling. Small specimens between 3 – 13 cm TL and juveniles were most abundant in the restored areas, except for *L. lota*, which was most abundant in the man-made bank riprap. Target species of conservation were mostly detected in restored areas, particularly the critical young life stages of *C. nasus*, *B. barbus* and *T. thymallus*. Water level strongly determined accessibility and suitability of bank habitats, with shallow, gravel-dominated habitats comprising flat bank angles being most beneficial for these species. The findings of this study provide evidence for the success of bank habitat restoration in structurally impacted alpine rivers on target species of conservation. Fluctuating water levels and discharges typical for alpine rivers should be better considered in restoration planning, particularly in light of climate change, affecting timing and amplitude of discharge in these systems.

9.2 Introduction

More than half of the world's large river systems are heavily modified and regulated (Dynesius & Nilsson, 1994) and therefore belong to the most threatened ecosystems worldwide (Ricciardi & Rasmussen, 1999). River channelisation and damming lead to longitudinal and lateral changes with respect to the flow regime as well as the river continuum (March & Fisher, 1999; Mueller et al., 2011; Pander & Geist, 2010). This results in negative impacts on the river ecosystem, as habitats and migration routes are altered and disturbed (Bunn & Arthington, 2002; Kondolf et al., 2006; Pander & Geist 2013). Consequently, habitat loss owing to anthropogenic impacts is considered one of the most severe limitations for the recovery of riverine fish faunas (Dudley & Platania, 2007; Stanford et al., 1996). The lack of shallow gravel banks and bank habitats is particularly critical for the early life stages of rheophilic species (Aarts et al., 2004; Grift, 2001; Keckeis et al., 1997; Pander et al., 2017; Schiemer et al., 2002), which comprise many species that faced strong population declines in the last decades (Aarts et al., 2004; Dudgeon et al., 2006; Mueller et al., 2018). Therefore, the accessibility and functionality of structurally diverse bank habitats for shelter, spawning and foraging during changing water levels is of great importance (Junk et al., 1989; King et al., 2009). Target species of conservation are of particular interest for restoration success owing to their complex life

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cycles and specific habitat preferences during certain life stages. Consequently, restoration measures targeting the quality enhancement of spawning grounds (Nagel et al., 2020b; Pander et al., 2015a; Sternecker et al., 2013b; Taylor et al., 2019; Zeh & Dönni, 1994), juvenile habitats (Lorenz et al., 2013; Pander et al., 2017), as well as structural features to prevent catastrophic outdrift during floods (Pander & Geist, 2018; Schiemer et al., 2001; Schludermann et al., 2012) are most popular. Past straightening of rivers often homogenised the entire bank zones of rivers, thereby creating uniform current conditions and rapidly deteriorating flow conditions during floods. Consequently, bank restoration in rivers typically targets increasing flow current diversity also including zones of low current that are mandatory for weak swimmers (Muhar, 1996; Schiemer et al., 2001) such as the larval stages of almost all target species of conservation. In addition, habitats for various life stages should be connected and located in direct vicinity to enable movement of larvae and juveniles among them (Pander & Geist, 2013).

The importance of bank habitat restoration in regulated rivers has been highlighted by several studies, but most of them focussed either on small-scale restorations (few meters shoreline) (Pander et al., 2017) or lowland rivers (Meulenbroek et al., 2018a; Ramler et al., 2016; Ramler & Keckeis, 2019; but see exceptions such as Wyżga et al., 2012). Studies assessing the effects of large-scale bank restoration in rivers with an alpine catchment, characterised by rapid and high fluctuations in discharge and water levels, are underrepresented in the scientific literature. Transferring findings from systems with different hydrographs and temperature regimes to alpine rivers is problematic.

The large alpine River Inn is a heavily impounded river with stabilised banks for flood protection and hydropower use. It is currently subject to a large restoration programme aiming at improving the quality and connectivity of habitats for its fish fauna. A major part of the restoration work has a focus on the restoration of bank habitats by removing the former bank stabilising structures to enable dynamic riverbank development.

This study investigates the seasonal and water level-dependent fish habitat use of altered and restored bank habitats in the River Inn to deduce evidence-based implications for future restoration programs (Geist & Hawkins, 2016; Jähnig et al., 2011; O'Neal et al., 2016). Bank restoration measures (less than 2 years after implementation) were compared with altered

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bank habitat types most commonly used for bank stabilisation, in particular bank riprap and concrete profile. Specifically, we hypothesised that (i) structural properties of bank habitats reveal strong seasonal differences induced by water level changes. (ii) These changes are reflected by changes in fish community composition. (iii) Restored bank habitats comprise highest species diversity and number of individuals and that (iv) target species of conservation *Hucho hucho*, *Barbus barbus*, *Chondrostoma nasus*, *Lota lota* and *Thymallus thymallus* are most abundant due to their distinct live cycle strategies largely depending on a high degree of lateral connectivity.

9.3   Material and Methods

9.3.1 Study area

This study was conducted at the River Inn, a formerly braided river (www.geoportal.bayern.de/bayernatlas, Pander et al., 2021). Nowadays, this river comprises a straightened single channel intensively used for hydropower generation (run-of-river power plants) with embankments for flood protection. Despite the heavy morphological alterations, the River Inn still comprises a natural hydrograph characterised by low discharges and a clear water phase in winter and, due to its glacier runoff, enhanced discharges of turbid and cold water in summer (Figure 37). In this river, water abstraction does not play a major role.

Table 17: Comparison of the discharge values for the study areas Feldkirchen, Wasserburg, and Ering (www.hnd-bayern.de)

Site	NW $[m^3s^{-1}]$	MW $[m^3s^{-1}]$	HW $[m^3s^{-1}]$	Summer sampling $[m^3s^{-1}]$	Autumn sampling $[m^3s^{-1}]$
Feldkirchen	104	316	1280	725	241
Wasserburg	131	357	1470	467	241
Ering	240	715	2740	901	512

Abbreviations: HW, mean flood discharge at least once annually reoccurring; MW, mean discharge; NW, mean low water discharge

To test for the seasonal fish habitat use in different bank habitats, three impoundment sections along the river course were selected as study areas covering a total stretch of ~125 km. Two were located in Upper Bavaria, in the districts of Rosenheim (47°50'17'' N, 12°09'12'' E), and Wasserburg (48°14'41'' N, 13°00'23'' E) and one in Lower Bavaria in the district of Rottal-Inn (47°57'38'' N, 12°09'26'' E) (Figure 38). The three bank habitat types concrete profile (CP), bank riprap (BR) and naturally restored riverbank (RR) were compared (Figure 39) across different seasons and water levels (Table 17, Figure 39).

Each bank habitat type was assessed with the same amount of replicates (6 replicates) in each of the three impoundment sections resulting in a total of 18 replicates for CP, BR and RR, respectively. CP and BR represent the heavily modified pre-restoration status. Such structures were analogously used for bank stabilisation in many other rivers worldwide (Chou & Chuang, 2011; Richardson et al., 2001). The already established restoration measures were located at a maximum distance of ~ 2,000 m and a minimum distance of ~ 700 m downstream of the hydropower plants. Restored riverbanks were investigated in spatial proximity (min. ~ 150 m, max. ~ 400 m) to the two other bank habitat types.
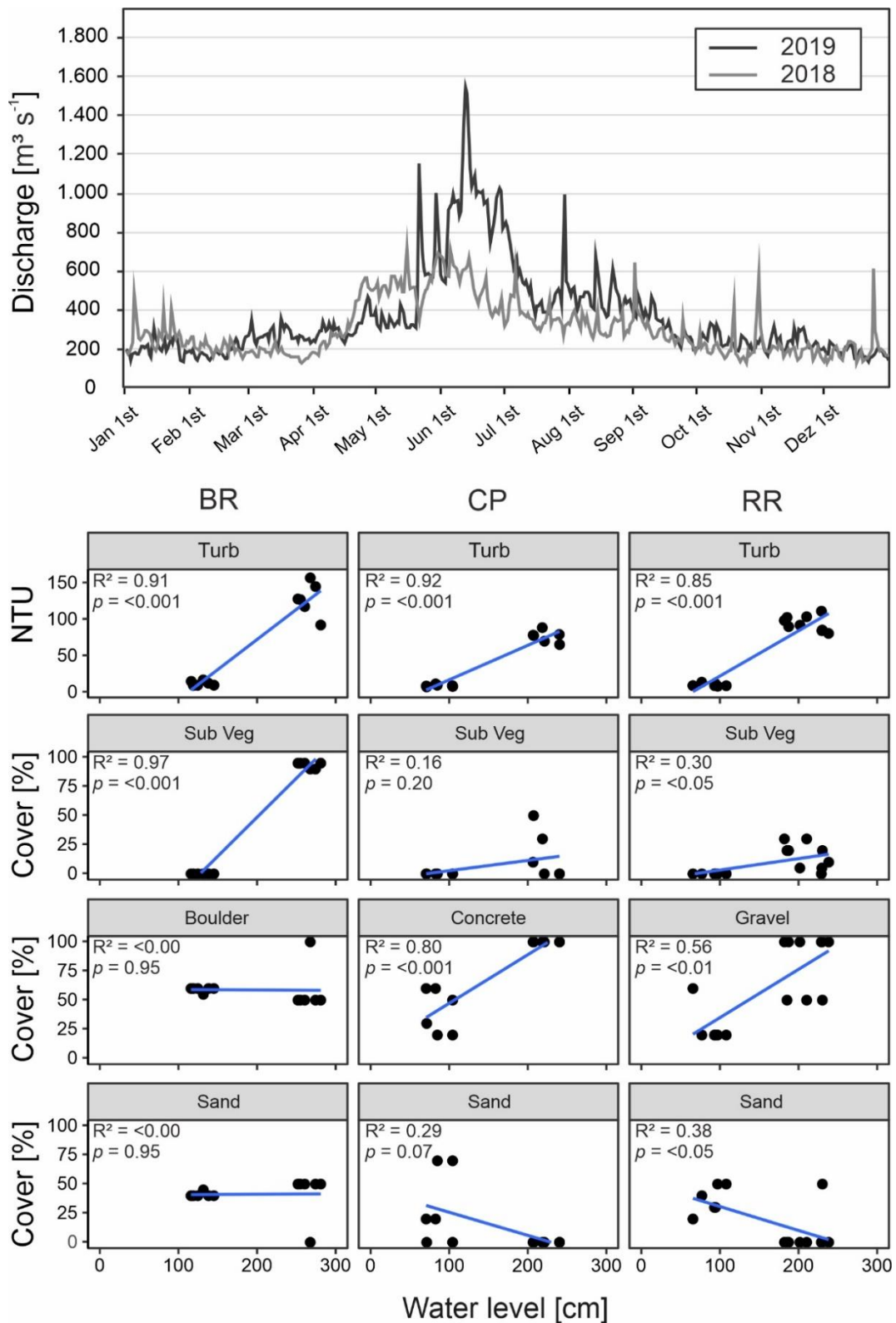
Figure 37: Hydrograph of the River Inn for the years 2018 and 2019, exemplarily shown for the Feldkirchen site (upper part of figure) and linear regressions of structural parameters in relation to water level change and bank habitat type (lower part of figure) measured at both sampling dates summer and autumn at the Feldkirchen site. Parameters include Turb, Sub Veg, and R, and the proportion of the bed material boulder, concrete, gravel, and sand cover. BR, bank riprap; CP, concrete profile; R, roots; RR, restored riverbank; Sub Veg, proportion of submerged vegetation (VEG); Turb, turbidity (NTU)

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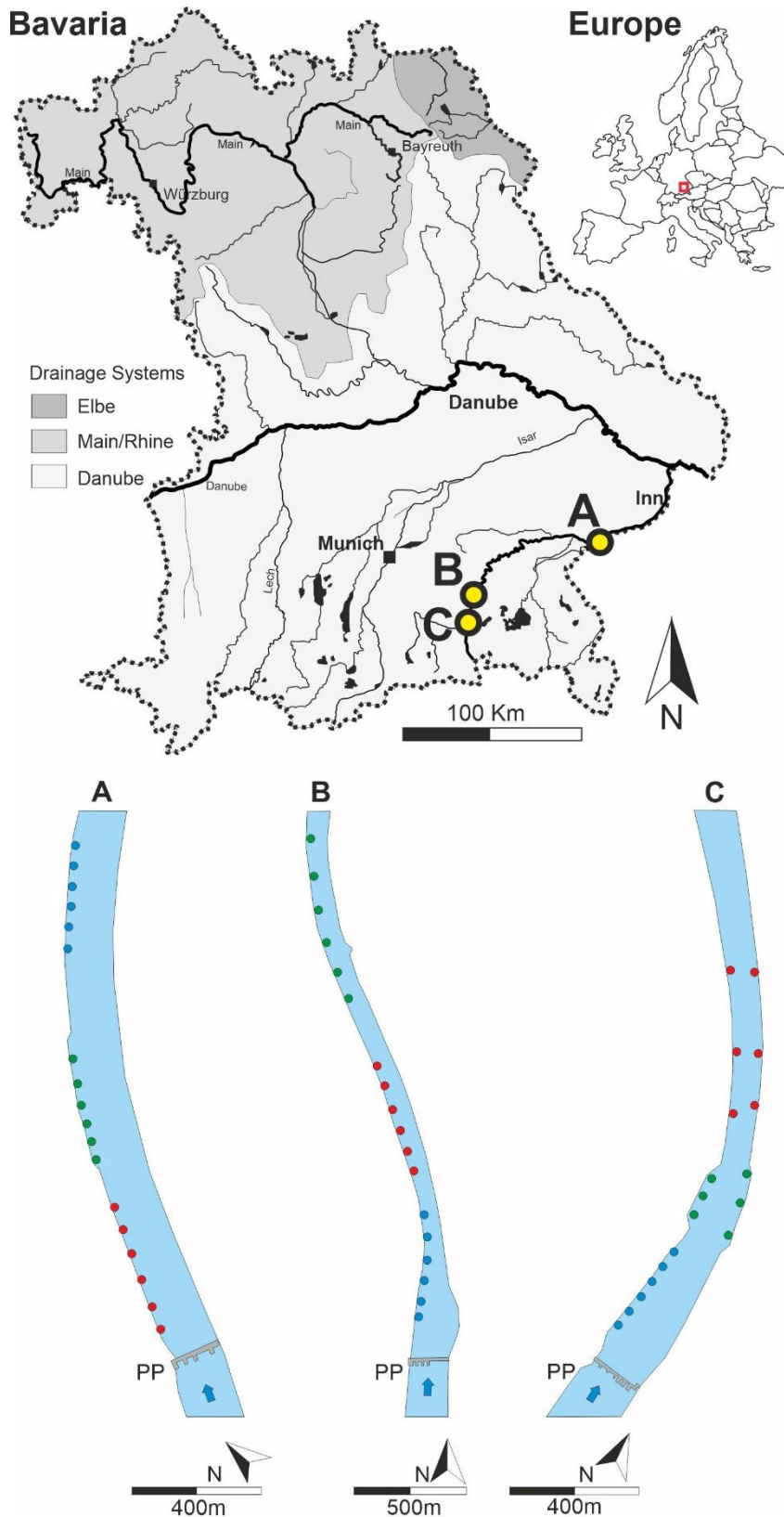
Figure 38: Map and magnification of the study area with the three major drainage systems of Bavaria (Germany) and the location of the study site within Europe (red rectangle). Yellow dots = location the three studied impoundments (A) impoundment Ering, (B) impoundment Wasserburg, (C) impoundment Feldkirchen. PP: green dots = restored riverbank, blue dots = bank riprap, red dots = concrete profile, and blue arrow = flow direction. PP, power plant

9.3.2 Bank habitat types

RR were implemented 1-2 years prior to the sampling 2019 and were present with variations in structural enrichment and vegetation cover. Variation 1 consisted of flattened banks and small islands creating small flow areas next to the main channel, intended to mimic small site branches of the formerly braided river course. Variation 2 was similar, except for the absence of islands (Figure 39). The gravely substrate of this habitat type was interspersed with individual or scattered granite boulders of the former bank riprap. The riparian vegetation consisted mainly of grass and small shrubs (*Salix spec.*), overhanging trees or larger shrubs were widely absent.
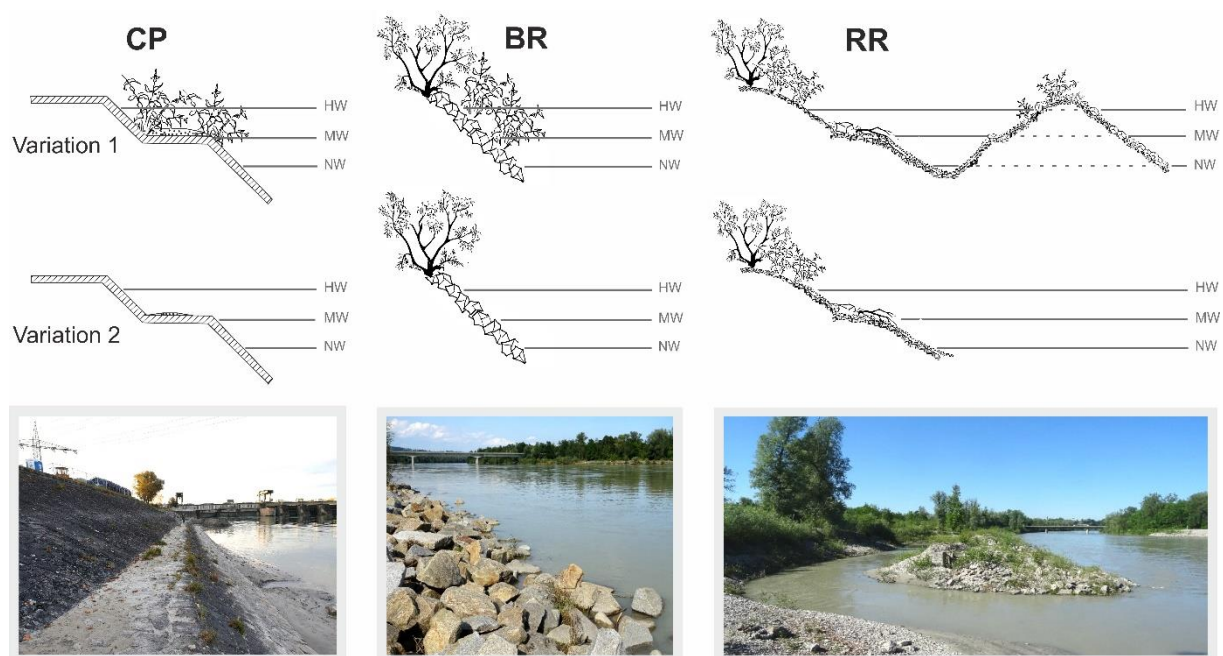
Figure 39: Schematic view of the three assessed bank habitats. CP, variation 1 with rich vegetation and variation 2 with poor vegetation; BR, variation 1 with rich vegetation and variation 2 with poor vegetation; RR, variation 1 with small islands in front of the main stream and variation 2 without small islands. BR, bank riprap; CP, concrete profile; HW, annual reoccurring flood; MW, mean annual flow indicating conditions during summer sampling; NW, low flow indicating conditions during autumn sampling; RR, restored riverbank

BR habitats were formerly introduced as an embankment constructed from granite boulders (diameter 77 cm – 140 cm) without the addition of concrete or steel. This construction design creates cavities and a very rough bank profile. BR was found in the variation (Figure 39) with rich overhanging riparian vegetation like trees, shrubs and tall forbs covering the whole bank

length or with poor vegetation (variation 2) consisting out of sparingly found small shrubs (*Salix ssp., Alnus ssp.*) and forbs (e.g. *Solidago gigantea, Impatiens glandulifera*).

CP is a hard bank revetment made of paving stones poured in concrete (Figure 39). The concrete profile consists of two slopes with an angle of inclination of 45° and a berme in between. Comparable to BR, the profile can have either rich vegetation (variation 1) or poor vegetation (variation 2). The berme can be covered with different loads of sand, creating the basis for the potential vegetation cover.

9.3.3 Abiotic habitat characteristics

Physicochemical habitat variables were measured according to Pander et al. (2017), with measurements taken in the middle and at the upper and lower end of each investigated stretch. Temperature T [C°], dissolved oxygen O2 [mgL^{-1}], electric conductance EC [μScm^{-1} related to 20°C] and pH [pH] were recorded with a handheld multi-meter (Multi 3430, WTW, Weilheim, Germany) 20 cm below the water surface. Turbidity [NTU] was determined with the handheld TURB 350 IR (WTW, Weilheim, Germany) at three measurement points within each stretch and with three replicates at all measurement points. Flow velocity [ms^{-1}] was measured, each with three replicates (beginning, middle and end of the sampling area) by magnetic-inductive flow measurement with the Ott Mf Pro (HFA Messinstrumente, Kempten, Germany) 10 cm above substratum (CSB) and 10 cm below surface (CSS). Water depth [cm] was determined with the same instrument. Additionally, the relative composition of river bed material was visually estimated in 5% steps in the field according to the classification block stone (B), concrete (C), gravel (G) and sand (S). In addition, wet sieving verified sediment box-samples (Pander et al. 2015a) from each habitat type in the laboratory. Second, the proportion of bank vegetation (VEG), dead wood (DW), submerged living roots (R) and canopy cover (CC) was also documented in 5% steps (see Pander & Geist 2018).

9.3.4 Fish sampling

Electrofishing was performed in the year 2019 according to the method of Pander & Geist (2010) at 2 m distance from the bank. It has to be noted that due to the differences in bank angle the electrofishing was carried out in different water depths potentially leading to a catch bias. However, water depth did not exceed critical depths where catch efficiency may be reduced significantly. Each stretch had the same length of 30 m, with 18 replicates per habitat type and season. Electrofishing was carried out during daytime in summer from July 31st to August 12th and in autumn from October 14th to October 16th. The three habitat types were evenly distributed over the three assessed impoundments (Figure 38). In order to ensure that always the same 30 m stretches were fished throughout the different sampling seasons, all of them were mapped with a hand-held GPS and marked in the field. Electrofishing (15 kW, EFKO-Fischfanggeräte GmbH, Leutkirch, Germany) was conducted with one hand anode and a dip net (mesh size 3 mm), which were always handled by the same persons. Fish were kept in plastic tanks with fresh water supply. After species determination, total length (TL) of each fish was measured to the nearest cm.

9.3.5 Statistical analysis

All multivariate analyses were computed with the statistical program PRIMER (Plymouth Routines in Multivariate Ecological Research, version idR v7, Auckland New Zealand). PCA analyses (Principal Components Analysis) based on Euclidean distance were used to assess differences of habitat conditions using abiotic habitat variables as listed in Supporting information 5. The PCA allowed an overlay with the measured variables indicating the strength of correlation to the arrangement of habitat types in the ordination plot. Environmental variables were standardised using the normalize function in Primer for environmental variables and visually checked for co-linearity. Variation of the habitat parameter turbidity as well as the proportion of submerged vegetation, boulder (only in BR), concrete (only in CP) and gravel (only in RR) and sand cover in relation to seasonally changing water levels were visualised using the package "ggplot2" in R (R Core Team, 2017). To detect significant relationships, linear regression analyses were calculated in R (version 3.6.3; R Core Team,

2020) using water level as a predictor and the respective habitat variables as response variables.

Number of species and number of individuals are given as total numbers detected in the sampling stretches and in relation to the overall catch of the study and compared between different sampling times and habitat types. Since all sampling stretches had the same bank length of 30 m no further standardisation was applied to the catch data.

To assess fish community composition in the respective sampling stretches, a Bray-Curtis-Similarity resemblance matrix based on differences in fish abundance data between season and habitat was generated and visualised by nonmetric multidimensional scaling (nMDS Clarke et al. 2014). In addition, the number of individuals of target species in sampling stretches (*Chondrostoma nasus*, *Barbus barbus, Lota lota, Thymallus thymallus and Hucho hucho*) was visualised using the bubble function. To account for zero values, dummy variables were computed as recommended by Clarke et al. (2014) as a standard procedure in PRIMER. ANOSIM (Analysis of Similarities) was then used to test for significant differences of fish community composition as well as abiotic habitat conditions between seasons and habitat types. To model relations between environmental and fish community data, distance based linear modelling (DistLM; Legendre & Anderson, 1999) was chosen, since this approach allows partitioning of explained variance and therefore a quantification of the relative influence of different habitat variables. To visualise these variables in the nMDS plot, the overlay function in Primer was used. The SIMPER (Similarity Percentages) analysis was used to identify the abiotic variables and the fish species contributing most to the similarity between seasons and habitats (Pander et al., 2015b). For all statistical analyses, significant differences were accepted at $p \leq 0.05$.

9.4   Results

9.4.1 Habitat characteristics

The PCA analysis of the abiotic habitat parameters (explained variability PC1 = 26.5% and PC2 = 18.6%) revealed a pronounced difference between all habitat types and seasons (Figure 40,

ANOSIM: Global R = 0.57, p < 0.001, pairwise comparisons Table 18). As evident from the arrangement of sampling stretches in the ordination plot along the PC1 axis, strong seasonal differences in habitat conditions dependent on discharge were evident, particularly in relation to turbidity, temperature, dissolved oxygen and electric conductance. From summer to autumn sampling, temperature (summer: 14.5 ± 0.7, autumn: 10.3 ± 0.5) and especially turbidity (summer: 121 ± 44, autumn: 12 ± 5) decreased distinctly over all habitat types, in contrast to higher values recorded for electric conductance (summer: 213 ± 17, autumn: 337 ± 27), and oxygen concentration (summer: 9.6 ± 0.4, autumn: 10.4 ± 0.1; for details see Supporting information 5). In addition to these parameters, SIMPER analysis (Table 18) detected high contributions to seasonal differences in the structural properties of submerged bank vegetation in BR (summer: 77 ± 22), and submerged roots in CP (summer: 1 ± 3), both falling dry and thus being completely absent during low water levels in autumn. Moreover, current speed was higher during high flow conditions in summer. Considering the river bed material, sand cover increased from summer to autumn in all habitat types (CP: +171%, BR: +13%, RR: + 56%) and correspondingly a lower share of the initial bed material, concrete in CP (-59%), bank riprap in BR (-11%), and gravel in RR (-59%), was documented (Supporting information 5). Over all habitat types, variability of habitat parameters was higher in summer during elevated discharge compared to the low-flow situation in autumn as indicated by the different variations of habitats in Figure 39.

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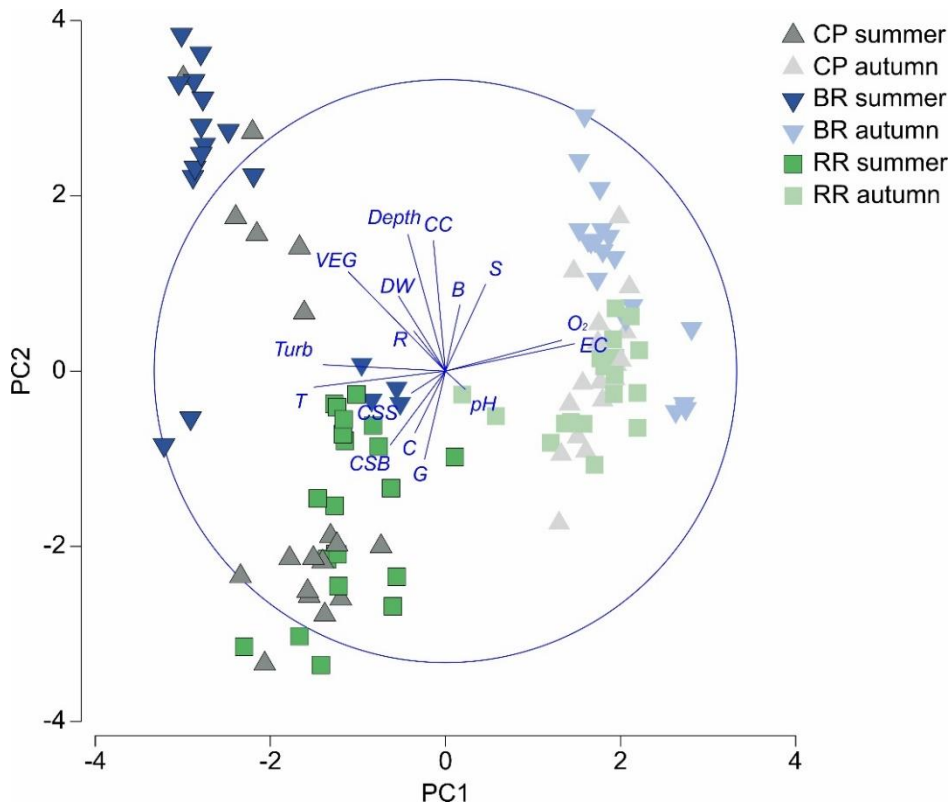
Figure 40: PCA of abiotic habitat parameters with all stretches, displayed by indicating the seasonal sampling. PC1 explains 26.5%, PC2 18.6% of the differences. The length of the vectors corresponds to the strength of the correlation (blue circle = 100%). The colored symbols represent the habitat types CP = grey, BR = blue, and RR = green, respectively for summer dark color and autumn light color. B, boulder in % coverage; C, concrete in % coverage; CC, canopy cover in % coverage; CSB, current speed 10 cm above substratum (m s^{-1}); CSS, current speed 10 cm below surface (m s^{-1}); Depth, water depth (cm); DW, deadwood in % coverage; EC, electric conductance standardised to 20°C in micro-Siemens per centimeter; G, gravel in % coverage; O2, oxygen concentration (mg L^{-1}); PCA, principal component analysis; pH, pH value; R, roots in % coverage; S, sand in % coverage; T, temperature (°C); Turb, turbidity as nephelometric turbidity unit; VEG, bank vegetation in % coverage

Dissimilarities between habitat types were mainly caused by inherent differences in terms of bed material (largely expressed along PC2), like the high share of concrete in CP (68 ± 32), bank riprap in BR (69 ± 22), or natural substrate such as sand (43 ± 30) and gravel (38 ± 34) in RR. Besides, the bank habitat types mainly differed in water depth (highest in BR and lowest in RR), current speed (highest in CP, lowest in BR), and the presence of canopy cover (highest in BR, lowest in RR), submerged bank vegetation (highest in BR, lowest in RR) or roots (highest in CP, absent in RR; Table 18, Figure 39; Supporting information 5.

Table 18: Group comparisons of the abiotic data according to habitat types and sampling seasons

	Group comparison	Av. squared	Ranked parameter contribution [%]			ANOSIM	
			1st	2nd	3rd	R	p
Habitat	CP vs. BR	36.84	B [11.34]	C [11.0]	R [8.0]	0.282	**<0.001**
	CP vs. RR	29.13	C [14.0]	G [11.8]	S [8.4]	0.219	**<0.001**
	BR vs. RR	37.96	B [9.4]	G [9.4]	CC [8.8]	0.312	**<0.001**
Season	CP_aut vs. CP_sum	31.36	R [13.3]	EC [11.3]	T [10.9]	0.669	**<0.001**
	BR_aut vs. BR_sum	44.44	VEG [13.2]	O$_2$ [11.2]	T [10.0]	0.660	**<0.001**
	RR_aut vs. RR_sum	30.42	Turb [12.4]	EC [12.2]	T [11.8]	0.606	**<0.001**
Habitat + Season	CP_sum vs. BR_sum	44.76	C [15.6]	B [12.3]	R [11.7]	0.468	**<0.001**
	CP_sum vs. RR_sum	35.19	C [19.8]	G [16.8]	R [11.8]	0.437	**<0.001**
	RR_sum vs. BR_sum	46.09	G [12.1]	CSS [11.9]	VEG [10.7]	0.536	**<0.001**
	CP_aut vs. BR_aut	12.59	B [23.0]	CC [23.0]	Depth [11.2]	0.482	**<0.001**
	CP_aut vs. RR_aut	9.19	CSB [17.6]	DW [15.2]	S [13.7]	0.213	**<0.001**
	RR_aut vs. BR_aut	14.06	CC [23.7]	B [16.3]	CSB [14.4]	0.462	**<0.001**

Note: Average (Av.) squared distance and parameter contribution (ranked after their contribution) is based on the results of the similarity percentages (SIMPER) analysis. R- and p-value are based on the analysis of similarities (ANOSIM)

Abbreviations: B, boulder in % coverage; BR, bank riprap; C, concrete in % coverage; CC, canopy cover in % coverage; CP, concrete profile; CSB, current speed 10 cm above substratum (m s^{-1}); CSS, current speed 10 cm below surface (m s^{-1}); Depth, water depth (cm); DW, deadwood in % coverage; EC, electric conductance standardised to 20°C in micro-Siemens per centimeter; G, gravel in % coverage; O2, oxygen concentration (mg L^{-1}); R, roots in % coverage; RR, restored bank; S, sand in % coverage; T, temperature (°C); Turb, turbidity as nephelometric turbidity unit (NTU); VEG, bank vegetation in % coverage

9.4.2 Fish habitat use

Over all sampling stretches and seasons a total of 2,536 individuals (including 7% target species) from 10 families and 30 fish species were caught during this study (Supporting information 6). The number of individuals was slightly higher in autumn (1,398) compared to the summer sampling (1,138) and the size spectrum of fish ranged from 1 - 86 cm TL, with 76% of the fish being ≤ 10 cm and 96% of the specimens being ≤ 15 cm (Table 19).

Overall species composition of the River Inn was dominated by cyprinids (87.1% of all specimens caught) and cottids (4.9%), with all other families contributing < 1% each. Among cyprinids, 78.2% were indifferent, 21.7% rheophilic and < 1% stagnophilic according to the flow guild classification by Zauner & Eberstaller (1999). Rheophilic species were most abundant in RR (15 species) and least abundant in BR (12 species). Stagnophilic species were only detected in CP and BR (one species each).

Table 19: Overview of the biotic results according to habitat types and sampling seasons

Season/Habitat	S	N	Target species	TL [cm]	Shannon
CP					
Autumn	20	204	BB, CN, LL	6.8 ± 5.7 [2-46]	2.04
Summer	18	240	BB, CN, LL, TT	11.3 ± 6.4 [2-45]	1.12
BR					
Autumn	21	571	BB, CN, LL	6.2 ± 4.1 [1-55]	1.77
Summer	16	430	BB, CN, LL	10.8 ± 5.1 [4-68]	1.05
RR					
Autumn	23	623	BB, CN, LL, TT	7.8 ± 6.2 [1-86]	2.25
Summer	17	468	BB, CN, HH, LL, TT	10.9 ± 7.0 [2-70]	1.16

Abbreviations: BB, Barbus barbus; BR, bank riprap; CN, Chondrostoma nasus; CP, concrete; HH, Hucho hucho; LL, Lota lota; N, number of individuals caught; RR, restored bank; S, number of species caught; Shannon, calculated Shannon diversity; TL, total length of all individuals caught given as mean ± SD [minimum to maximum]; TT, Thymallus thymallus
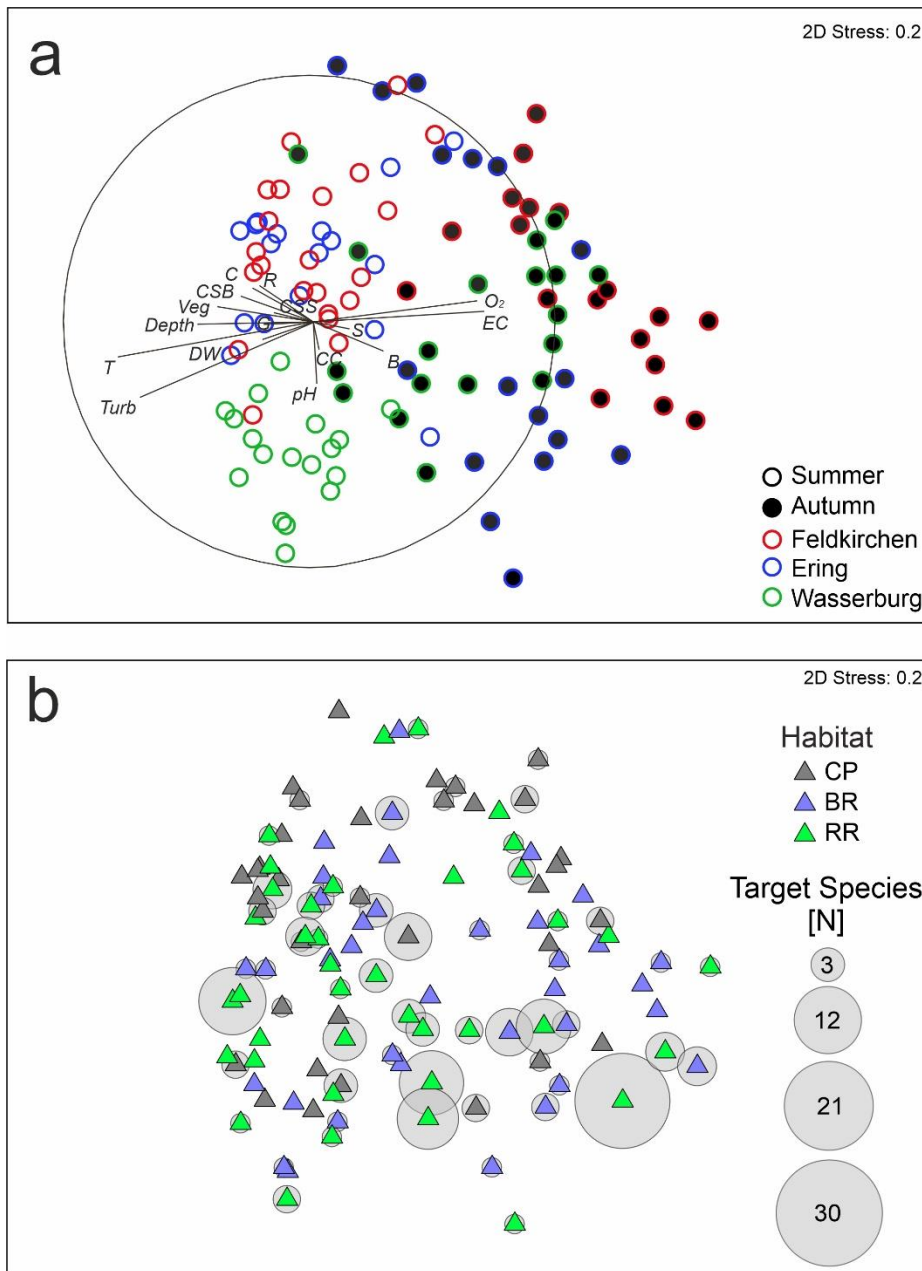
Figure 41: NMDS of fish community composition based on fish-abundance data in the three different habitat types sampling seasons based on Bray-Curtis-Similarity, with each symbol representing a distinctive sampling stretch. The distance between the circles (sampling stretch) corresponds to the similarity in fish community composition (small distance = large similarity). The two parts of the figure indicate (a) seasonal differences and differences between impoundments and (b) differences between habitat types and number (N) of target species displayed as bubbles (*Chondrostoma nasus*, *Barbus barbus*, *Lota lota*, *Thymallus thymallus*, and *Hucho hucho*). The size of the bubbles corresponds to the number of target species caught in the respective habitat. The colored symbols represent the habitat types concrete (CP, grey color), bank riprap (BR, blue color) and restored (RR, green color). B, boulder in % coverage; C, concrete in % coverage; CC, canopy cover in % coverage; CSB, current speed 10 cm above substratum (m s^{-1}); CSS, current speed 10 cm below surface (m s^{-1}); Depth, water depth (cm); DW, deadwood in % coverage; EC, electric conductance; EC, standardised to 20°C in micro-Siemens per centimeter; G, gravel in % coverage; NMDS, nonmetric multidimensional scaling; O2, oxygen concentration (mg L^{-1}); pH, pH value; R, roots in % coverage; S, sand in % coverage; T, temperature (°C); Turb, turbidity as nephelometric turbidity unit (NTU); VEG, bank vegetation in % coverage

Table 20: Group comparisons of the biotic data according to habitat types and sampling seasons

	Group comparision	Av. dissimilarity	Species contribution						ANOSIM	
			AA	AB	CG	CN	GG	SC	R	p
Impoundment	Ering - Wasserburg	72.28	37.46	8.40	-	-	3.66	11.90	0.313	**<0.001**
	Ering - Feldkirchen	71.09	16.89	10.69	7.90	4.57	-	19.82	0.141	**<0.001**
	Feldkirchen - Wasserburg	74.97	36.37	Apr 88	-	-	-	19.29	0.230	**<0.001**
Habitat	CP - BR	82.00	32.68	11.42	7.42	-	-	24.48	0.084	**< 0.01**
	CP - RR	81.51	36.39	9.04	-	5.52	6.66	16.03	0.049	**<0.05**
	BR - RR	79.29	32.51	9.47	4.93	-	-	23.41	0.026	0.105
Season	CP aut - CP sum	85.66	36.64	11.48	10.82	-	-	16.73	0.165	**< 0.01**
	BR aut - BR sum	86.70	30.22	11.90	-	-	-	31.72	0.568	**<0.001**
	RR aut - RR sum	87.04	33.35	7.49	-	5.99	6.31	19.07	0.489	**<0.001**
Season + Habitat	CP sum - BR sum	75.57	56.63	5.91	6.80	-	-	6.29	0.035	0.148
	CP sum - RR sum	72.06	60.00	6.39	-	5.62	-	-	0.081	**<0.05**
	RR sum - BR sum	66.51	58.19	-	5.54	5.34	-	5.44	0.002	0.340
	CP aut - BR aut	80.24	10.00	17.16	7.40	-	-	38.72	0.242	**<0.001**
	CP aut - RR aut	83.11	13.00	12.25	9.34	6.11	7.50	25.48	0.087	**<0.05**
	RR aut - BR aut	76.39	9.50	14.48	5.07	-	6.19	34.98	0.071	0.052

Note: Average (Av.) dissimilarity and species contribution are based on the results of the similarity percentages (SIMPER) analysis. R- and p-values are based on the analysis of similarities (ANOSIM). Significant differences are highlighted in bold

Abbreviations: AA, Alburnus alburnus; AB, Alburnus bipunctatus; BB, Barbus barbus; BR, bank riprap; CG, Cottus gobio; CN, Chondrostoma nasus; CP, concrete profile; GG, Gobio gobio; RR, restored bank; SC, Squalius cephalus

Multivariate analysis of fish community composition revealed highly significant seasonal differences (ANOSIM: Global R 0.291, p < 0.001; Figure 41a, Table 20). Differences in fish community composition among habitat types were more pronounced in autumn than in summer, except for the pair BR – RR, where differences in autumn were only significant at p = 0.01 (Table 20). In contrast to the strong seasonal turnover, differences in fish community composition within habitat turnover were less pronounced (ANOSIM: Global R 0.052, p < 0.05; Figure 41b, Table 20). Significant differences in fish community composition were found among CP – BR and CP – RR, but not between BR and RR (Table 20). DistLM identified significant correlations of physicochemical habitat variables (e.g. Turb, EC, O_2 and pH) and structural variables (e.g. Depth, CSB, G, C, S, DW, VEG and roots, Table 21) with the fish community composition as visualised in the nMDS (Figure 41). The SIMPER-analysis detected a set of six species (*Alburnus alburnus*, *Alburnus bipunctatus*, *Cottus gobio*, *Chondrostoma nasus*, *Gobio gobio* and *Squalius cephalus*) that mainly contributed (more than 70%) to the differences (dissimilarity) in community composition, between the sampling seasons and habitat types likewise. Out of this set of species, *A. alburnus*, *A. bipunctatus* and *S. cephalus* constantly contributed to the dissimilarity of habitats. *A. alburnus* (2-fold) and *S. cephalus* (4-fold) were more abundant in BR than in CP and *A. alburnus* (2-fold), *S. cephalus* (3-fold) and *C. nasus* (5-fold) showed a higher abundance in RR compared to CP. Additionally, *C. nasus*, a species with high conservation value in this river, contributed strongly to the seasonal differences in RR being 2.5-fold more abundant in autumn compared to summer sampling (Figure 42).

Table 21: Results of the distance-based linear modeling (DistLM) for the relations between environmental and fish community data

	R^2	Proportion	Pseudo-F	p-value	df
Depth	0.056	0.056	6.492	**<0.001**	109
CC	0.110	0.054	6.506	>0.05	108
C	0.127	0.017	2.079	**<0.05**	107
B	0.140	0.014	1.687	**<0.01**	106
EC	0.208	0.067	8.898	**<0.001**	105
G	0.224	0.016	2.154	**<0.01**	104
pH	0.256	0.032	4.424	**<0.001**	103
S	0.256	0.000	NA	NA	103
O$_2$	0.279	0.024	3.336	**<0.001**	102
CSS	0.284	0.004	0.622	>0.05	101
CSB	0.298	0.014	1.997	**<0.001**	100
T	0.347	0.049	7.406	**<0.001**	99
DW	0.350	0.003	0.500	**<0.05**	98
Turb	0.413	0.063	10.342	**<0.001**	97
VEG	0.420	0.007	1.203	**<0.001**	96
R	0.431	0.011	1.812	**<0.01**	95

Note: Significant differences are highlighted in bold

Abbreviations: B, boulder in % coverage; C, concrete in % coverage; CC, canopy cover in % coverage; CSB, current speed 10 cm above substratum (m s⁻¹); CSS, current speed 10 cm below surface (m s⁻¹); Depth, water depth (cm); df, degrees of freedom; DW, deadwood in % coverage; EC, electric conductance standardised to 20°C in micro Siemens per centimeter; G, gravel in % coverage; O2, oxygen concentration (mg $^{L-1}$); pH, pH value; Proportion, proportion of individual variable on the overall correlation; Pseudo-F, multivariate analogue of Fisher's F ratio; p value, significance accepted at p < 0.05; R, roots in % coverage; R2 , cumulative proportion of explained variation for the model; S, sand in % coverage; T, temperature (°C); Turb, turbidity as nephelometric turbidity unit (NTU); VEG, bank vegetation in % coverage
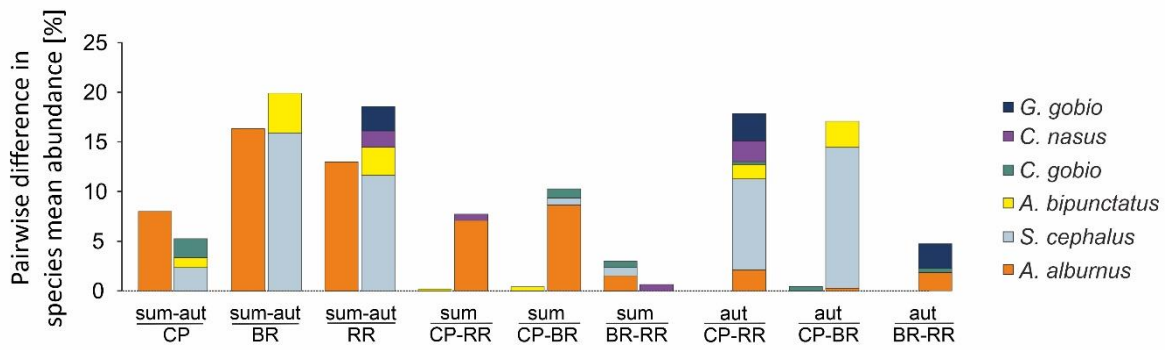
Figure 42: Results of the SIMPER analysis between the habitat types CP, BR, and RR in the seasons sum and aut. Bars indicate the summed up absolute difference of the mean abundance per habitat and season of the six fish species that contribute most to the dissimilarity between habitat types. aut, autumn; BR, bank riprap; CP, concrete profile; RR, restored riverbank; sum, summer

In all habitats a relatively large number of species was recorded, with most species caught in CP (a total of 26 species) compared to RR (25 species) and BR (23 species). The recorded number of individuals was highest in RR (total of 1,091 individuals), with 14% more individuals caught during autumn compared to summer sampling. In BR, also a higher number of individuals (1,001 individuals) was caught during autumn (14%). This is in contrast to the results in CP (444 individuals) where 8% more individuals were caught in summer.
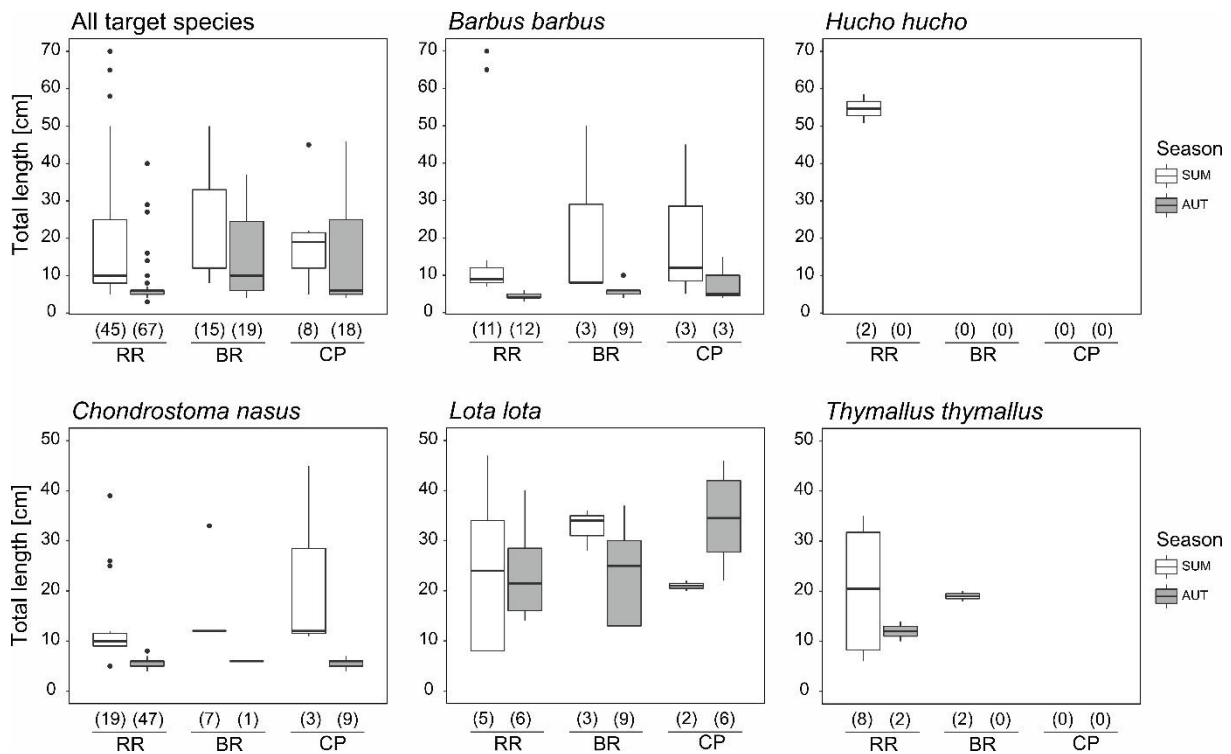
Figure 43: Length distribution of target species (top left), *Barbus barbus* (top middle), *Hucho hucho* (top right), *Chondrostoma nasus* (bottom left), *Lota lota* (bottom middle), and *Thymallus thymallus* (bottom right) in the

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three habitat types CP, BR, and RR and for the sampled seasons summer and autumn. Numbers in brackets below boxes = number of individuals. BR, bank riprap; CP, concrete profile; RR, restored riverbank

In this study, target species were documented with an overall of 172 specimens (7% of all fish caught), from which *C. nasus* (86 individuals) was most abundant, followed by *B. barbus* (41 individuals), *L. lota* (31 individuals), *T. thymallus* (12 individuals) and *H. hucho* (two individuals). The rheophilic cyprinids *C. nasus*, *B. barbus* and the only gadid species *L. lota* used all bank habitat types. In contrast, *T. thymallus* was solely found in BR and RR habitats. The highly endangered and due to its high trophic position naturally rare *H. hucho*, was only found with low numbers in RR in summer. These differences in habitat use among species were also evident considering specific size classes of target species. During summer sampling, *C. nasus* and *B. barbus* were caught in all sizes ranging from < 10 cm to > 40 cm (Figure 43). In contrast, these species were exclusively documented in the size class < 10 cm and in higher numbers during autumn sampling (*C. nasus*: summer = 29, autumn = 57; *B. barbus*: summer = 17, autumn = 24), indicating greater habitat suitability for this sensitive life stage. *L. lota* was strongest represented by individuals > 10 cm.

9.5   Discussion

As highlighted in this study, bank habitat quality and restoration success in alpine rivers are strongly determined by seasonal changes of water levels. Hydrographs of these systems are predicted to be strongly altered by climate change with potential consequences on the quality of fish habitats. For instance, an earlier onset of the glacier- and snowmelt (Bard et al., 2015) as well as increased magnitudes of discharge are expected when glacier melt and heavy summer rains coincide (Braun et al., 2000). Long-term scenarios additionally predict a further change in alpine hydrographs towards a distinct reduction of summer discharge (Braun et al., 2000) and rising water temperatures (Hari et al., 2006). Many studies highlighted the importance of bank habitat restoration in rivers of different degrees of anthropogenic modification (e.g. Guzelj et al., 2020; Hirzinger et al., 2004; Pander & Geist, 2010; Pander & Geist, 2016). Whilst restoration of lowland rivers has been intensively covered (e.g. Erős et al., 2008; Ramler & Keckeis, 2019; Stoffers et al., 2021), this is to the best of our knowledge the first study comparing restoration of bank habitats in a large, high-gradient alpine river.

Whilst restoration in both, lowland and alpine rivers is often directed towards a similar set of measures, the differences between these river types in flow and temperature regimes cause differences in habitat function throughout the year. All bank habitats in the River Inn comprised large differences in their structural characteristics due to the general change of water level (up to 1.32 m during the study intervals) caused by an annually recurring process triggered by glacier and snowmelt. This yearly change in water level strongly affects abiotic habitat conditions of BR and CP and leads to strong seasonal differences in habitat availability and quality, since structures such as riparian vegetation, dead wood and roots were only fully submerged at high discharge, creating additional habitat space for shelter and feeding (Rozas & Odum, 1988). In RR, the changes in habitat quality and space were different, since two years after restoration only scarce bank vegetation was present compared to the other habitats. Due to the higher water level in summer, the constructed small side-channels in RR were completely flooded providing additional aquatic habitats resembling small branches of the former braided river (Pander et al., 2021) as indicated for variation 1 of this habitat type. Braided, anabranching river courses are nowadays scarce in Europe, yet recent evidence from the large and near–natural Vjosa River (Albania) demonstrates the high ecological value of river types with such a dynamic hydromorphology (Schiemer et al., 2020). Current speed above ground was higher in summer leading to reduced sand depositions. In turn, the general bed material of the habitat types was exposed, as evident in the higher shares of visible concrete in CP, boulder stones in BR and gravelly substrate in RR, potentially favoured by lithophilic fish species.

Fishes are known to be fast colonisers of new aquatic habitats as long as those are fully connected, particularly when living conditions change seasonally (Pander et al., 2015b; Pires et al., 1999). Considering the abiotic differences between habitats and particularly seasons and results derived from lowland rivers (Erős et al., 2008; Meulenbroek et al., 2018a; Ramler & Keckeis, 2020), a large difference in overall fish community composition between CP, BR and RR as well as a strong seasonal change of community composition was expected. Surprisingly only the latter was observed in this alpine river and the strong seasonal differences detected were mainly attributed to higher species richness and numbers of individuals in autumn, except for CP where more fish were caught in summer. The set of species detected by SIMPER indicated strong seasonal habitat preferences of *A. alburnus*

(much more abundant during summer) and *S. cephalus*, *A. bipunctatus*, *C. gobio*, *G. gobio* and *C. nasus* being mostly present in the bank habitats during autumn. Depending on the individual species, the observed abundance shifts can result from habitat use change, recruitment (particular in autumn season as most caught species spawn in spring or early summer) as well as a combination of both, matching the observed times of spawning and subsequent larval drift (Nagel et al., 2021b). Since some of those species are ubiquitous schooling fish (e.g. *A. alburnus*), their occurrence can be dependent on distinct pattern of habitat use according to reproduction, food availability and predator density (Pitcher, 1986; Brown et al., 2011) being strongly governed by seasonality (Pander & Geist, 2010). In contrast, *C. nasus* is a rheophilic specialist and it is well-known that particularly the juvenile size classes, most frequently caught in this study, have a distinct preference for shallow gravel bank habitats (as can be found in RR) due to diet shifts in their early ontogeny (Pander et al., 2017; Jurajda, 1999). The recorded fish lengths in the bank habitats were rather small compared to the overall fish inventory of the river, which clearly indicates that these habitats were predominantly used by juvenile life stages or small fish species. In addition, strong seasonal differences of the mean total length were observed in all habitat types, with larger fish being caught during summer sampling (up to 4.6 cm difference) with elevated water levels and higher shares of submerged vegetation, compared to autumn sampling where these structural properties were completely absent. This is surprising, since many authors who investigated fish habitat use in rivers concluded that structure holds particularly small fish (e.g. Pander & Geist, 2010) and therefore those were expected to hide or feed in the flooded vegetation of BR and CP during summer sampling. During high flow conditions in summer, the flow velocity in CP and BR increased also due to their steep bank angle and the associated limited potential for lateral expansion of the water volume. As a result, the retention capacity for biota in the bank zone, as explained by Schiemer et al. (2001), was severely reduced, leading to unfavourable current conditions particularly for juvenile size classes and small fish species. Apparently, this occurred irrespective of the available submerged bank vegetation or roots linked with the elevated water level. In RR, the higher water level in summer created a larger habitat area due to the more flattened bank angle and the fully flooded side channels. However, the current speed increased here as well as in the other habitats. Our results indicate that the higher shares of gravel in combination with high current speed created

habitat conditions favouring rheophilic species, which were most frequently caught in this habitat. This was particularly evident when considering the target species of this study, which were mostly caught over the shallow gravel banks of RR. It is well-known that gravel banks with high current are used for spawning in spring by the rheophilic target species *B. barbus* (e.g. Melcher & Schmutz, 2010), *C. nasus* (Nagel et al., 2020b) *H. hucho* (Esteve et al., 2013) and *T. thymallus* (Zeh & Dönni, 1994). Moreover, it is likely that the restored bank habitats may also be beneficial for foraging of this species, irrespective of their high variability in feeding strategies. These comprise benthic feeding on macroinvertebrates and small fish in the gravelly substrate of riffles (*B. barbus*: Baras & Cherry, 1990), grazing of phytobenthos growing on large stones (*C. nasus*: Gerke et al., 2018) as well as drift-feeding on macroinvertebrates emerging from the river bed (*T. thymallus*: Watz et al., 2014). In addition, stomach content analysis of the piscivorous *H. hucho* revealed that a large proportion (~ 40%) of the diet composition consists of the three rheophilic target species mentioned above (Šubjak, 2013), which could explain the co-occurrence of these species detected in RR. The flow current-sensitive life stages of larvae and young juveniles also find habitat space in RR, particularly in autumn by retreating to the very shallow bank line where less current pressure occurs. In addition, shallow bank zone with strongly reduced current warm up faster than the main stem, favouring the development of phyto- and zooplankton (Schiemer et al., 2001) which provides an important food source for these life stages (e.g. Reckendorfer et al., 2001).

In contrast, the generalist *L. lota* which is also considered a target species in the River Inn was caught in slightly higher numbers in BR compared to RR. This can be attributed to the species nocturnal life strategy and the resulting preference to cavities of large stones (Eick, 2013), widely available in BR. Besides hiding places, BR habitats may also provide several food sources for this species such as macroinvertebrates, fish eggs, as well as larval and juvenile fish, in particular of speleophilic species (Hares et al., 2015). Therefore, *L. lota* tend to live in the largest possible rocky outcrops, which would also appear in a natural alpine river, where feeding for this species seem to be most efficient (Fischer, 2000). Similar habitat preferences for riprap structures have also been described in invasive gobies (Brandner et al., 2015), which have been documented in the Bavarian Danube, but not yet in the entire course of the River Inn.

9. Water level induced changes of habitat quality determine fish community composition in restored and modified riverbanks of a large alpine river

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9.6   Conclusions

Bank habitat restoration of highly modified alpine rivers is a barely investigated subsection of worldwide studies in the field of river restoration. Since alpine rivers are among the most heavily modified ones originally harbouring a distinctive set of highly specialised species, it is necessary to assess restoration measures in the context of a high degree of disturbance. In particular, the improvement of the lateral connectivity as implemented in the restored bank habitats is of great importance, because it allows flat bank angles, which result in shallow water zones and areas of less current speed. Those areas quickly warm up during summer and provide distinct refugia facilitating fast growth of young fish in the otherwise cold and harsh environment particularly in this alpine river. The strong effects of fluctuating discharges governing habitat quality and habitat use by fish in the River Inn points to the necessity of better considering these specific properties in restoration management. This holds particularly true in light of climate change affecting melting of glaciers and the long-term changes in timing and magnitude of alpine discharges that have been predicted.

10. General Discussion

The conservation of freshwater ecosystems and their biodiversity is one of the greatest and most urgent challenges for human civilization in the 21st century (Dudgeon et al., 2006). For decades the complexity of stream ecosystems was greatly simplified and conservation strategies were based on single charismatic species or even just single life stages (De Leo & Levin, 1997; Geist, 2011). In recent years, integrative and holistic approaches have been proposed, such as the ecosystem based management (EBM; O'Higgins et al., 2020) or the concept of integrative freshwater conservation (Geist, 2011). These sophisticated management strategies value the complex multidimensionality as well as hierarchical organisation of stream ecosystems and their biodiversity and set the general framework and guidelines for successful conservation. To make conservation strategies a success, however, it is equally important to investigate specific conservation measures and their abiotic and biotic effects within this framework. To this end, it is essential to fill knowledge gaps in the ecology of threatened freshwater fish species, many of which still exist (Smialek et al., 2019).

This thesis synthesises the results from six scientific case studies related to conservation efforts of *Chondrostoma nasus*, ranging from the genetic to the ecosystem scale. The outcomes of this thesis contribute important ecological knowledge on this species, which fills previously existing knowledge gaps. By systematically analysing different conservation tools and monitoring techniques, they also provide specific guidance for practitioners, who are in charge of implementing conservation measures in the field. The case studies presented in this thesis are embedded in a holistic and integrative framework, which considers the multidimensionality of stream ecosystems and the complex nature of freshwater biodiversity. Based on the findings obtained, future conservation strategies of *Chondrostoma nasus* and freshwater fish in general can be set more systematically and evidence-based.

The assessment of an ongoing supportive breeding programme of *Chondrostoma nasus* provided important evidence that the current approach (see Harsanyi & Aschenbrenner, 1995) of just using a very limited number of spawners can severely affect the genetic diversity of the offspring. It further revealed that not all remaining populations are equally suited as a source for supportive breeding measures, which demonstrates the need to assess genetic parameters prior to the implementation of such initiatives (Chapter 4). Supportive breeding is

a common and increasingly used conservation tool to support declining populations or to reintroduce species where they have been expatriated (Lamothe & Drake, 2019). However, for species with a high genetic exchange rate during spawning such as *Chondrostoma nasus*, a repatriation approach, in which spawned and fertilised eggs from the wild are collected, may better represent the genepool than stripping a very limited number of spawners (Stoeckle et al., 2022).

The life of a fish starts with the release of eggs during spawning and from this time onwards it is exposed to the natural environment and potential disturbances (Fuiman & Werner, 2009), highlighting the need to fill knowledge gaps in this very first step of the life cycle. A case study on egg properties of different *Chondrostoma nasus* populations in the River Inn catchment revealed intraspecific differences in the egg surface structure of freshly laid eggs, which likely explain previously observed differences in egg adhesiveness and hatching success between these populations (Chapter 5). This study was the first time SEM imaging, a commonly applied technique to study interspecific differences in fish eggs (Riehl & Patzner, 1998; Rizzo et al., 2002), was used to determine intraspecific differences in egg properties of nase. This study further demonstrated that the newly developed assessment matrix provides a useful tool for identifying intraspecific differences in fish species with adhesive eggs when combined with multivariate analysis tools (Nagel et al., 2021a).

Promptly after spawning, the eggs of *Chondrostoma nasus* interact with the gravel bed by adhering at the substrate surface, although the quality of the spawning gravel was thought to have only minor relevance for successful egg development (Keckeis et al., 1996). To challenge this view, a combined approach of a laboratory (Chapter 6) and field experiment (Chapter 7) was used to systematically study the faith of spawned eggs of *Chondrostoma nasus* in relation to the spawning substrate quality. These studies revealed that hatching is much more successful in spawning substrate with no or only minor shares (<10%) of fine sediment and further showed that hatched larvae stay in the sheltering interstitial zone for up to two weeks until emergence (Nagel et al., 2020ab). Such evidence was previously only known for species of the salmonid family and was not expected for substrate-spawning cyprinids such as *Chondrostoma nasus*. Both studies greatly highlight the previously overlooked importance of substrate quality and vertical connectivity at spawning grounds of *Chondrostoma nasus* and provide important guidance for future restoration planning to support recruitment of this

species. This is of particular importance since functional gravelly spawning grounds are among the habitats most vulnerable to the degradation of river systems (Geist & Hawkins, 2016; Mueller et al., 2011). The field experiment also revealed that spawning adults prefer restored substrate areas to not restored ones at similar conditions of water depth and flow velocities, indicating that *Chondrostoma nasus* can determine such differences in substrate quality (Nagel et al., 2020b).

After hatching and successful emergence from the streambed, fish larvae drift downstream, which provides an essential path for colonisation and gene-flow (Lechner et al., 2016; Pavlov, 1994). However, larval drift is heavily impacted by hydropower use and damming of rivers (Humphries et al., 2002; Pavlov et al., 2020), making it necessary to look for mitigation measures to restore safe drift corridors in such altered water bodies. To systematically assess the drifting larval fish community in a heavily modified waterbody and a restored fish bypass system, spatio-temporal drift patterns were studied in the large alpine River Inn, including those of *Chondrostoma nasus*. This study revealed a clear seasonal chronology of larval drift for *Chondrostoma nasus* and several other endangered species of the fish community in this river. In addition, a clear shift to nocturnal drift in almost every fish species was observed (Nagel et al., 2021b). These findings provide an important first basis to direct turbine and water management in hydropower affected rivers in a more "fish larvae-friendly" way, e.g. by adjusting turbine management in relation to peak movement times, or by strengthening alternative drift corridors other than turbine or spillway passage. The spatial comparison of drift samples taken along a downstream gradient in a nature-like fish bypass further revealed that these restoration measures can not only provide important drift corridors but also functional spawning grounds for several endangered species of the rheophilic fish community, including *Chondrostoma nasus*. This finding lines in a growing body of evidence on the habitat function of these types of fish passes (Meulenbroek et al., 2018b; Pander et al., 2013; Tamario et al., 2018) and impressively highlights their ecological value, besides the aspect of solely restoring habitat connectivity. Finally, this study also proofed the need of obtaining unambiguous results in the taxonomic identification of fish larvae and eggs, e.g. by applying DNA barcoding, especially in environments with a highly diverse fish community, since morphometric identification alone is often not precise (Ko et al., 2013; Lira et al., 2022; Meulenbroek et al., 2018ab)

In the later ontogeny, larvae of riverine fish species, exit the drift and use bank zones for further development (Pander et al., 2017; Stoffers et al., 2022). However, most river banks are straightened and stabilised with boulders and concrete, eliminating habitat diversity and thereby important ecological features (Bunn & Arthington, 2002). To gain insights in the potential of bank habitat restoration in heavily modified large waterbodies, a study compared three bank habitat types in the River Inn systematically and seasonally, including heavily modified and restored banks, and assessed the use of these habitats by the fish community. The results of this study showed that the restoration of riverbanks in large alpine rivers can be successful as it provides important habitat heterogeneity and thereby favors the abundance of the rheophilic fish community, including juvenile *Chondrostoma nasus* (Pander et al., 2021), yet it has to proof its long-term functionality. This study also contributed important knowledge for the restoration planning of bank habitats in rivers with a highly fluctuating discharge, as it systematically assessed the change in habitat conditions caused by greatly changing water levels. The latter is becoming of increasing importance in light of climate change effects, which are predicted to heavily alter functional processes such as the flow regime of streams, particular in the alpine region (Jasper et al., 2004).

The basic principle for a comprehensive and successful conservation management of freshwater fish is the understanding of the life stage specific traits and relevant stressors (Geist, 2011; Geist, 2015). As the results of the six presented case studies demonstrate, the integration of different assessment methods and monitoring tools can reveal important new insights into the ecology of important target species, even in comparatively well-studied fish such as *Chondrostoma nasus* (see Smialek et al., 2019). Successful conservation management of freshwater fish must take into account the complexity of this task and therefore requires an integrative and holistic approach that encompasses all developmental stages in the life cycle of freshwater fish, the different levels of biodiversity organisation, and the hierarchical and multidimensional nature of river ecosystems. To this end, conservation managers must be aware of the strengths, limitations, and pitfalls of applicable conservation measures and monitoring techniques in their toolkit.

10.1   How to integrate all biodiversity levels in conservation planning – the suitability of selected conservation measures

In this thesis, field and laboratory studies were combined using conventional sampling methods along with advanced techniques to assess the applicability of different measures for freshwater fish conservation. The measures evaluated aim at a holistic conservation approach that embraces the whole life cycle of freshwater fish as well as the complex nature of biodiversity organisation and the multidimensionality of river ecosystems.

10.1.2 Ensuring genetic integrity and intraspecific variability of fish populations in supportive breeding measures

Supporting endangered wild populations by releasing individuals reared in captivity, known as supportive breeding, is an important tool in the conservation of freshwater fish (Ford, 2002; Wedekind, 2002). However, maintaining genetic integrity and intraspecific variability in such measures is critical, as this determines the potential for adaptation to shifting environmental conditions (Ford, 2002; Willi et al., 2007), and thus ultimately population survival. Given the increasing awareness of the freshwater biodiversity crisis, species with little commercial value such as *Chondrostma nasus* have recently also become targets of such programmes, in addition to already existing ones for commercially important species such as most salmonids (Blanchet et al., 2008; Marie et al., 2010). However, there is a lack of evidence in many initiatives on how these measures affect the genetic integrity of hatchery-sourced offspring and, subsequently, wild populations. At the same time, many studies report a risk of supportive breeding measures to wild populations which arises from an unappropriated selection of parental animals in captive environments (Campton 1995; Ford, 2002; Rymann et al., 1999). The evaluation of a supportive breeding programme for *Chondrostoma nasus* in the catchment of the Inn River in Chapter 5 of this thesis found similar evidence, as it demonstrated that the current approach of using just a very limited number of adult fish reduces allelic richness in the progeny while increasing signs of inbreeding (Stoeckle et al., 2022). Even though the negative effect of using only a few adults for supportive breeding could be particularly prone in species which are characterised by a high degree of genetic exchange

during reproduction, such as *Chondrostoma nasus*, the effect may also be visible in other species with a differing spawning strategies as a general rule of thumb suggested by Ryman et al. (1999) says to use at least 50 adults, irrespective of the spawning strategy of the targeted species. Another critical aspect is the sex ratio of adults used, which in theory should consider equal numbers of males and females (Klupp & Geist, 2018; Wedekind, 2002). In wild populations of *Chondrostoma nasus*, males are generally overrepresented (Lusk et al., 1995), yet it is unclear how many males actually contribute to reproduction. To avoid a skewed sex ratio by favoring one male in egg fertilisation during supportive breeding, Wedekind (2002) proposes to mix equal amounts of milt from several males before adding to the eggs.

The results in Chapter 4 further revealed that the genetic constitution of the offspring significantly differed between *Chondrostoma nasus* populations, which spawn only 5 km apart in the same catchment, although the genetic constitution of the spawning adults was very similar. This highlights the need to also include genetic analysis of wild progeny in the assessment of supportive breeding programmes, as recruitment problems of certain populations may otherwise be overlooked. Contrary to the high genetic similarity of the two observed spawning populations, high-resolution SEM imaging of the eggs surface structure in Chapter 5 detected distinct intraspecific variability between these two populations (Nagel et al., 2021b). This demonstrates that a holistic screening of the genetic and intraspecific structure of fish populations benefits from the use of a variety of tools. Despite a lack of final clarification whether genetic effects are causing the observed differences in egg quality, and whether they are detrimental or a positive sign of local adaptation, these findings demonstrate that even populations originating from the same catchment can show a pronounced level of intraspecific diversity. As such, it is prudent to use individuals from multiple populations for supportive breeding programmes, as this approach can maintain genetic and intraspecific diversity while reducing the risk of inbreeding effects (Lutz et al., 2021).

Although the assessment in Chapter 4 observed only a single year event, supportive breeding of *Chondrostoma nasus* in the catchment of the River Inn has been conducted for more than ten years and is expected to be continued. This approach is in line with recommendations of several other programmes, which demonstrated that the success of supportive breeding measures also relies on the maintenance of rearing activities over multiple years, to minimise

the risk of unintended impacts such as selection effects or genetic bottlenecks (Geist et al., 2021; Hess et al., 2012).

This thesis provides specific guidance for improve supportive breeding of *Chondrostoma nasus*, however, more research is needed to clarify (1) how many adults and which effective sex ratio is needed to best mimic the genetic exchange rate in the wild, (2) how long offspring should be raised in captivity before being released into natural habitats, and (3) which habitats are best suited as stocking sites.

Concluding the results obtained, the following approach is suggested to improve supportive breeding measure for *Chondrostoma nasus*:

1. Screening of potential source populations prior to supportive breeding measures, including genetic analysis and other high resolution tools to assess intraspecific diversity.
2. Implementation of parental mixing ratio most closely to the intraspecific diversity in the catchment and the natural spawning behavior, e.g. by using multiple adults from different populations and by following guidelines in the fertilisation process.
3. Constant screening of hatchery-sourced and wild progeny to identify necessary adjustments in the parental mixing ratio.
4. Maintenance of supportive breeding programmes over several years.
5. Strengthening the natural reproductive capacity by habitat restoration measures.

Ultimately, the value of supportive breeding as a conservation measure in general is controversial discussed in the international literature, ranging from a valuable tool to maintain and enhance weak populations (Hess et al., 2012) to a threat to genetic integrity of wild populations (Antognazza et al., 2016). Supportive breeding should therefore not be the only conservation measure to support populations of endangered fish species and should run in parallel with efforts to restore the natural reproductive capacity through habitat and catchment restoration.

10.1.2 The ecological value of stream habitat restoration to conserve freshwater fish biodiversity

The alteration of natural flow regimes and the associated loss and fragmentation of habitats has been identified as the major cause for the drastic decline of freshwater fish populations (Bunn & Arthington, 2002). Consequently, stream restoration is a fast-growing field in scientific and practitioner activity (Smith et al., 2014). This thesis demonstrates that stream habitat restoration, a widely used tool to conserve and enhance freshwater biodiversity, benefits from a holistic and systematic approach that addresses restoration at all levels of river connectivity along with the quality of important key habitats in the whole life cycle of riverine fish.

The early life stages of riverine freshwater fish show a high sensitivity towards environmental disturbances (Schiemer et al., 2002), which holds particular true for substrate-spawning fishes who rely on a loose stream bed for successful egg development (Taylor et al., 2019). However, fine sediment infiltration has in many places severely degraded these key habitats (Kondolf, 2000; Sear et al., 2008). Using different substrate compositions as predictors while hatching success and larval size at emergence as response variables, the results of the laboratory study in Chapter 6 revealed a high susceptibility of *Chondrostoma nasus* eggs and larvae to fine sediment infiltration on spawning grounds, which was likely underestimated in the past (Nagel et al., 2020ab). These results line in a broad body of evidence on the negative impacts of the stressor fine sediment on the development of eggs and larvae from species of the salmonid family (Boulton et al., 1998; Kemp et al., 2011; Sear, 1993). They also contribute important knowledge to the only recently recognised susceptibility of early life stages of rheophilic cyprinid species to fine sediment infiltration (Duerregger et al., 2018; Gutmann Roberts et al., 2020), highlighting the severe threat this stressor poses to a large number of substrate-spawning fish species. As these studies and the results of this thesis indicate, infiltration of fine sediments and the associated colmation and compaction of the stream bed can exert multiple negative effects on egg and larval development, ranging from oxygen depletion in the hyporheic zone (Greig et al., 2007) and physical clogging of micropores in the egg chorichon (Kemp et al., 2011) to blocking of connectivity for eggs that need to penetrate into the stream bed (Duerregger et al., 2018) as well as for larvae that must emerge from there back into the water column. In addition to the observed reduced hatching success and

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increased number of blocked larvae in substrates with elevated shares of fine sediment in Chapter 6, signs of early emergence were documented in these treatments (Nagel et al., 2020a). Although the long-term survival of these larvae seems questionable (Brännäs, 1995; Louhi et al., 2011), some studies also point out benefits of early emergence such as first residency in subsequent high-quality larval habitats (Einum & Flemning, 2000; Harwood et al., 2003). The observed negative effects of the stressor fine sediment on the critical early life stages of *Chondrostoma nasus* clearly highlight the importance of substrate quality and vertical connectivity in spawning areas of this species, which may aid efforts to restore this key habitat.

Figure 44: Illustration of impacted (left) and a functional (right) vertical connectivity at gravel spawning grounds in relation to the early life history of riverine fish species

For substrate-spawning fish such as *Chondrostoma nasus*, suitable spawning sites are naturally scarce, thus, degradation or loss of suitable spawning grounds can be critical for the survival of remaining populations (Taylor et al., 2019). To improve the quality and vertical connectivity of gravel spawning grounds several short-term measures are available, such as the cleaning of colmated gravel (Bašić et al., 2017; Shackle et al., 1999) or the introduction of gravel in various size classes (Knott et al., 2021; Pander et al., 2015a). Chapter 7 of this thesis investigates gravel cleaning with an excavator at two well-known spawning grounds, revealing that this restoration tool has an immediate positive effect on the quality of the gravel composition following the implementation of this measure (Nagel et al., 2020b). Gravel cleaning also positively reflected on the biotic endpoints spawning activity and emergence success, which demonstrates that the results obtained in a laboratory environment in Chapter 6 also account under realistic conditions in natural habitats. Although being a quick, cheap and effective

restoration measure, other studies revealed that gravel cleaning also comes with limitations, which include possible negative effects of the washed out fine sediment on downstream habitats and the lack of long-term endurance of this measure (Pander et al., 2015a). Thus, gravel cleaning or gravel introduction should not be the only strategy to support reproduction success of substrate-spawning fish. To achieve more sustainable effects, such measures should be integrated in a catchment restoration programme that also incorporates the restoration of river functional processes such as sediment dynamics (Auerswald & Geist, 2018) or the elimination of stressors at their source, e.g. the reduction of fine sediment washout by an appropriate land use (Davies et al., 2009; Knott et al., 2018).

Besides being affected by a hampered vertical connectivity, rivers around the globe are heavily fragmented by instream obstacles, particular in Europe where recent counts revealed a mean density of 0.74 instream barriers per kilometer (Belletti et al., 2020). This alteration of rivers has been closely linked to the declining population trends of migratory (both diadromous and potamodromous) fish species (Deinet et al., 2020). Restoration of longitudinal connectivity has therefore evolved to a key aspect in policies supporting river restoration programmes such as the WFD (Council of the European Communities, 2000) and is mainly conducted through the construction of bypass facilities, spanning from rather technical construction schemes to nature-like solutions (Larinier & Marmulla, 2004). However, all systems have in common that they are preliminarily designed with an emphasis on upstream movements of juvenile and adult life stages of certain target species (Larinier & Marmulla, 2004; Silva et al., 2018). As Chapter 8 of this thesis demonstrated, nature-like fish passes also provide important migration corridors for downstream drifting fish larvae, which appears to be a previously undervalued and overlooked feature of this restoration measure (Silva et al., 2018). In line with a study conducted in the River Danube (Meulenbroek et al., 2018b), the results of Chapter 8 further revealed that a large part of the fish community of the river used the investigated nature-like fish pass as a drift corridor in the larval stage, indicating that these types of passage ways allow downstream migration of fish larvae at the community scale (Nagel et al., 2021b). Although there is little and contrasting evidence on the injuries and mortality fish eggs and larvae face when migrating through turbine and spillway passage (Boys et al., 2016; Morgan, 1976; Pracheil et al., 2016), where they encounter the risk of blade strikes, shear-stress or rapid pressure changes (Morgan et al., 1976; Pracheil et al., 2016), it seems likely that downstream drift through fish passes provides a safer corridor. By facilitating

upstream and downstream migration of the fish at the community scale and in relation to all life stages, genetic exchange and thus intra- and interspecific diversity of fish populations is promoted. This holds also true for the completely passively (eggs) or partly passively (larvae) migrating early life stages, as drift of ichthyoplankton greatly contributes to the dispersal and thus the genetic exchange of fish populations (Lechner et al., 2016; Pavlov, 1994). However, this aspect has received surprisingly little attention in river restoration science (Brudvik, 2011). Restoring safe fish passage, with a focus on all life stages and in up- and downstream direction is particular important in heavily altered and fragmented river systems, where fish populations are at risk of becoming isolated into small subpopulations (Peacock et al., 2016).
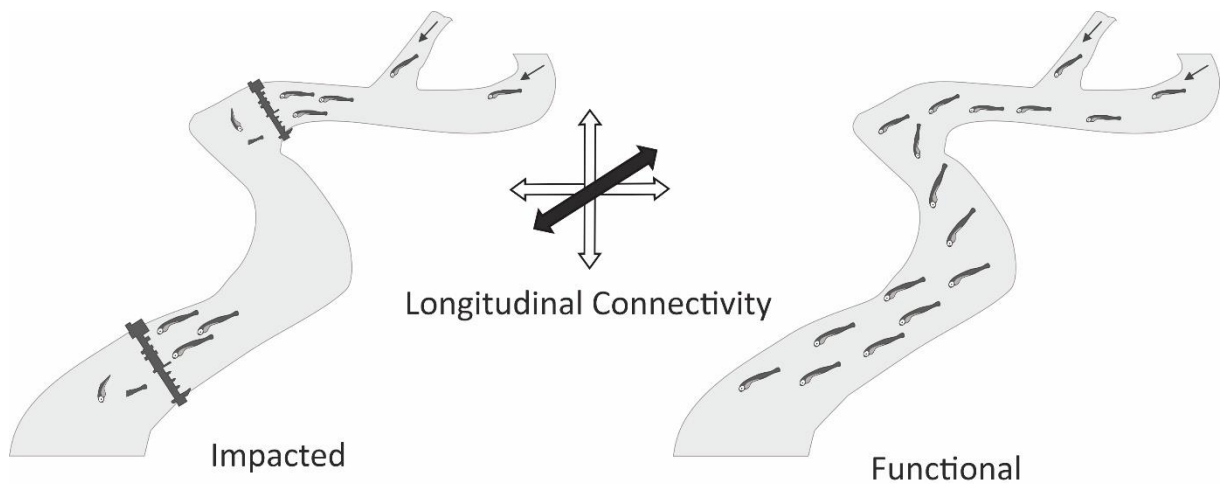
Figure 45*: Illustration of impacted (left) and functional (right) longitudinal connectivity in river systems in relation to the early life history of riverine fish species*

The study in Chapter 8 also observed that nature-like fish passes can provide functional spawning habitats for threatened and protected rheophilic fish species, and thus also contribute to habitat and community diversity (Nagel et al., 2021b). These findings line in a growing body of evidence on the ecological value of fish passes in nature-like construction schemes (Meulenbroek et al., 2018b; Nagel et al., 2022; Pander et al., 2013). Besides solely restoring longitudinal connectivity, such designs allow for the integration of habitat features for fish (Pander et al., 2021) and a broad range of invertebrate taxa (Gustafsson et al., 2013; Nagel et al., 2022), and thus ultimately contribute to an improvement in overall aquatic biodiversity. However, to maintain these features, nature-like fish passes need regular maintenance, since important functional processes such as discharge and sediment dynamics

are very restricted. Only recently, restoration planners aim to integrate river functional processes in these restoration measures, e.g. by applying discharge fluctuations (Meulenbroek et al., 2018b) in addition to sediment management plans (Mühlbauer et al., 2022). The integration of such aspects in the design and management of bypass facilities may mark an important step in the transition from a rather static approach in fish passage restoration, based on facilitating upstream migration of certain target species, towards more holistic and integrative concepts, which focus on the restoration of functional processes.

Another main issue in river restoration activity arises from the straightening of flow courses and the associated stabilisation and homogenisation of river banks (Bunn & Arthington, 2002). These alterations eliminate lateral connectivity between shallow bank zones in the floodplain and the main river (Tockner & Stanford, 2002). As a consequence, 90% of all floodplains in Europe are functionally extinct and 79% of the riparian areas of European rivers are considered "cultivated" (Tockner & Stanford, 2002).

The results in Chapter 9 revealed that even relatively simple measures of bank habitat restoration, like removing former stabilisation structures, can exert positive effects on the fish community, particular on endangered rheophilic species such as *Chondrostoma nasus* (Pander et al., 2021). Consistent with a broad range of other studies (see Stoffers et al. (2021) for a comprehensive review), this thesis further demonstrates that restored bank habitats are particularly important as nursery habitats for young-of-the-year fish when they show shallow water depths, slow-flowing to stagnant conditions and a variety of substrates, including gravel. In such conditions, water warms up more quickly compared to the main stem, featuring the production of food sources and larval growth (Balcombe et al., 2007; Nunn et al., 2007). Such habitat attributes are needed by larval and early juvenile life stages as they show a limited swimming and prey capture ability (Flore & Keckeis, 1998). In addition, lateral connectivity can also improve the availability of refugia during floods (Schiemer et al., 2001), which is particular important in rivers with a highly fluctuating discharge, such as the River Inn, investigated in Chapter 9. Consequently, the availability of shallow bank habitats and the related lateral connectivity between the main stem and the floodplain is a fundamental prerequisite in the early life history of riverine fish.

The results of this thesis further demonstrate that bank habitat and floodplain restoration measures need to be embedded in an integrative catchment restoration concept, which

considers functional processes in rivers such as highly fluctuating discharge patterns and sediment dynamics (Serra-Llobet et al., 2022). Such processes may alter the quality and connectivity of restored habitats and may lead to unintended effects if not considered in the planning stage. This is particular important in light of climate change, which is expected to heavily change the current discharge patterns of rivers in temperate regions (Jasper et al., 2004).

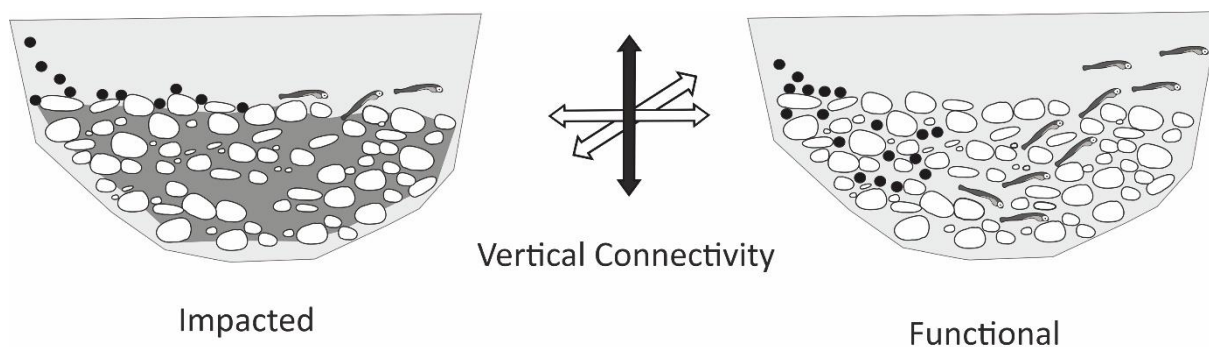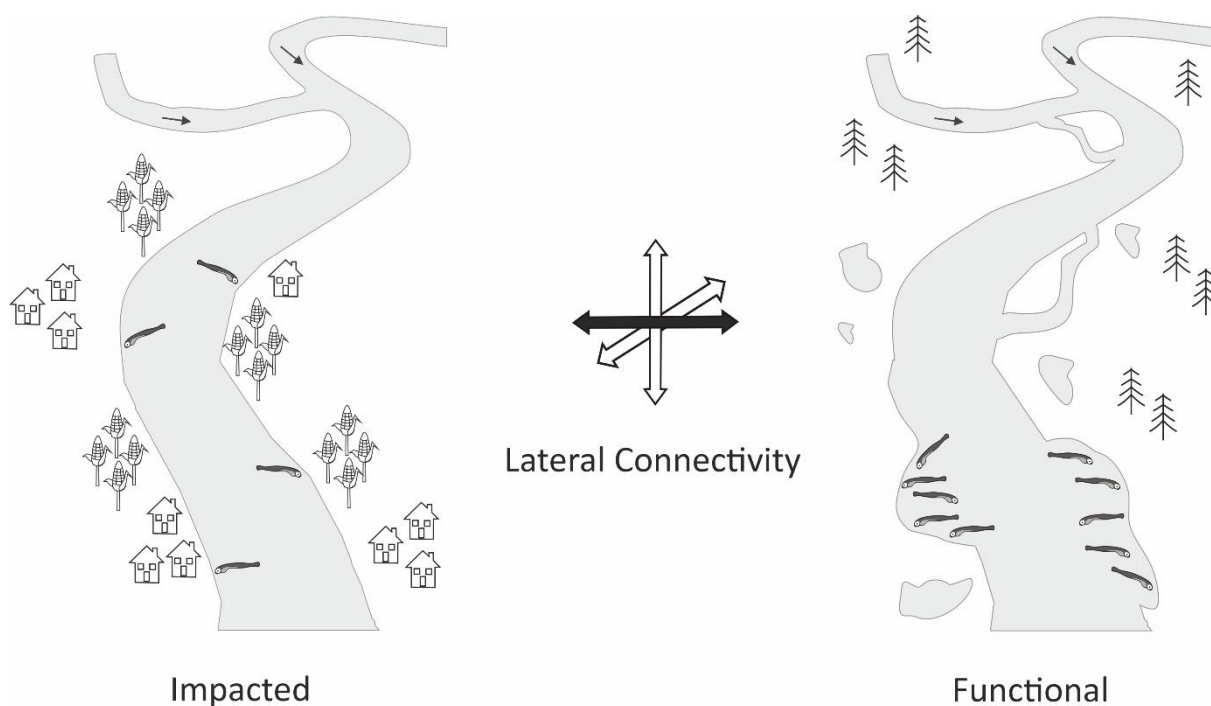**Figure 46**: Illustration of impacted (left) and functional (right) lateral connectivity in river systems in relation to the early life history of riverine fish species

The positive ecological effects observed in the restored bank habitats in Chapter 9 should not mask the fact that the investigated restoration measures should rather be understood as a local widening of the river bed and not an extensive restoration of the floodplain. In order to generate large-scale ecological effects, more extensive restoration measures are needed, such as those that have been implemented in other highly degraded rivers like the River Danube (Pander et al., 2015b; Stammel et al., 2012) and the River Rhine (Stoffers et al., 2021). Findings from these projects suggest that the best ecological effects are achieved when several complementary habitats are available in the floodplain, comprising various habitat characteristics and differing degrees of connectivity to the main stream (Pander et al., 2018; Stoffers et al., 2022). Such large-scale restoration measures can also restore functional

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processes such as nutrient, sediment, and discharge dynamics, at least to some extent (Stammel et al., 2012). However, implementing such projects is a very difficult task as they are severely constrained by legal and economic aspects, due to land use changes having widely eliminated former floodplain habitats by converting them to agricultural or residential areas (Schober et al., 2020).

10.2 Effective monitoring of freshwater fish conservation efforts – benefits and limitations of conventional and advanced technologies

Monitoring fish populations is a difficult but critical task in the conservation management of freshwater fish biodiversity as it provides mandatory information needed to evaluate the success or failure of conservation measures. Today, a wide range of technologies for monitoring of freshwater fish populations exists, greatly differing in cost and capabilities. A comprehensive assessment of all available techniques is beyond the scope of this thesis. The following elaboration therefore preliminarily focuses on techniques used in Chapters 4-9, with additional technologies being emphasised whenever appropriate. All the work conducted in this thesis was embedded in a large scale restoration and fish monitoring programme at the River Inn, which allowed an integrative and holistic monitoring of fish populations with different monitoring tools, targeting all life stages of freshwater fish at different levels of biodiversity organisation.

10.2.1 Monitoring genetic aspects of freshwater fish biodiversity

Genetic monitoring is an important part of effective conservation strategies for freshwater fish biodiversity as it provides valuable information on the genetic diversity and structure of a population (Schultz et al., 2022), e.g. in the context of supportive breeding measures. As evidenced by the results in Chapter 4, accompanying supportive breeding programmes with a genetic monitoring is key to avoid unintended effects such as loss of genetic variability and inbreeding effects (Stoeckle et al., 2022). This should ideally include a screening of the genetic structure and diversity of potential populations at the start of such programmes, to identify the extent of genetic and intraspecific variability in the catchment and thus the most

appropriate source populations for supportive breeding. Apart from genetic information on the parental fish, it is equally important to monitor the genetic constitution of wild- and hatchery sourced progeny of the targeted species in the due course as this can provide necessary information on unintended effects like inbreeding depression (Stoeckle et al., 2022). Such effects can be caused by an inadequate selection of parental fish as highlighted by the outcomes in Chapter 4. Finally, it is important to monitor the success of supportive breeding initiatives and their genetic effects in the long run, which usually requires a marking of the stocked fish. This can be done by different methods depending on the number and size of reared fish to be released into the wild. If stocking is done in larval or early juvenile life stages, large numbers can be tagged with mass marking techniques, e.g. using otolith marking with Alizarin red (Beckmann & Schulz, 1996), which allows a quick marking of large quantities of larvae, while having no known adverse effects on growth and survival (Lejk & Radtke, 2021). If the fish are reared to a larger size before stocking, marking techniques for an individual identification are applicable such as the use of PIT tags (Thorstad et al., 2013). Such marking techniques allow for an assignment of hatchery-sourced fish once they are recaptured, which aids in estimations about the contribution of stocking measures in the support of wild populations.

The application of genetic monitoring techniques can also be used to solve taxonomic uncertainties and thus contribute to an identification of invasive (Belle et al., 2017) or cryptic species (Bickford et al., 2007). This holds also true for the identification of the early life stages of fish (see Chapter 8), which often lack sufficient morphometric identification criteria (Lira et al., 2022). At the same time, sound taxonomic identification in the eggs and larvae is particularly important, as the presence of these life stages indicates successful spawning of a certain species in a given area, thereby providing valuable information for conservation managers. This clearly highlights the need of integrating high-resolution techniques in the taxonomic identification of fish eggs and larvae, e.g. by applying DNA barcoding. Although technical innovations in the last decades towards automated high-throughput machines have significantly reduced the time and cost of DNA barcoding, analysis of the entire community captured in ichthyoplankton surveys (often 5,000 - 30,000 individuals, see: Meulenbroek et al., 2018ab; Ramler et al., 2016) can still get very expensive. The approach of genetically verifying a sub-sample of pre-defined groups based on morphometric criteria, as suggested in Chapter 8 of this thesis, can provide an efficient trade-off between obtaining sound taxonomic

information while ensuring cost-efficiency. Recently, additional technologies have emerged (see Nayak et al., 2021), which may further ease correct species identification of fish larvae in the future. First evidence from the application of holographic imaging in larval fish determination reveals promising results and indicates that this technology may aid to correct taxonomic identification of fish larvae in the future when combined with machine learning (Nayak, 2021).

Finally, new advanced techniques allow for non-invasive monitoring of fish populations at the community scale, which will be discussed in the following section along with classic freshwater fish monitoring techniques.

10.2.2 Holistic monitoring of freshwater fish communities

Monitoring tools for freshwater fish communities are generally classified in capture-independent and capture-dependent techniques (Lucas & Baras, 2000; Radinger at al., 2019). Capture-independent monitoring techniques include hydroacustics and visual observations and recently also the collection of environmental DNA (eDNA) samples (Lucas & Baras, 2000; Wang et al., 2021). Visual observations of fish populations can be done from the shore or boats, by snorkeling, diving, or using camera-systems (Lucas & Baras, 2000). While such methods allow relatively undisturbed observation of fish behavior, major limitations arise in deep or turbid waters, such as those often found in the River Inn, the main study area of this thesis (see Chapters 8, 9). However, visual counting of spawning *Chondrostoma nasus* from the shore of the Mangfall and Sims River in Chapter 7 revealed robust results on the number and microhabitat use of these fish on spawning grounds. Yet, recent studies indicate that technological advances in the field of unmanned aerial vehicles (UAVs) combined with remote sensing may provide a powerful alternative for the monitoring of spawning activities of fish, at least in shallow and clear waters (Ponsioen et al., 2021; Sviridov et al., 2022)[3].

The collection of shed cellular material through water samples, known as environmental DNA (eDNA), is another rapidly emerging technology in the field of capture-independent monitoring techniques (Goldberg et al., 2016). Such approaches can be used for different

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[3] https://www.youtube.com/watch?v=BUNJp-fQn9Y (UAV based video of spawning *Chondrostoma nasus* in the River Mangfall taken during the 2017 spawning season; last accessed 20 January 2023)

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taxonomic groups in aquatic ecosystems, although fishes dominate eDNA studies in the scientific literature (Belle et al., 2019). Approaches of this monitoring technique reach from a holistic monitoring of the fish community in whole stream ecosystems (e.g. as already applied in the Danube catchment; Pont et al., 2023) to the search of single very rare or invasive species (Meulenbroek et al., 2022; Thomas et al., 2020). Although eDNA sampling provides an advancing, cost-effective and non-invasive sampling technique, the latter being particular important in light of an growing awareness of fish welfare matters (Browman et al., 2019), existing limitations of this method must be considered. These particular include constraints of information on species abundance and the lack of information on the size and demography of a population (Belle et al., 2019; Stoeckle et al., 2016). However, as this monitoring technology continues to evolve, it will likely provide a strong complement to traditional detection methods (Radinger et al., 2019).

Capture-dependent sampling techniques for freshwater fish monitoring require the capture of fish to allow species identification, the collection of biometric data (e.g. length, weight; see Chapter 8 & 9) and scales or tissue samples (see Chapter 4) (Radinger et al., 2019). Such data are necessary for estimations about the taxonomic and demographic structure of fish communities. Capturing freshwater fish is usually conducted with electrofishing and net- or trap-based sampling techniques (Bonar et al., 2009; Lucas & Baras, 2000; Radinger et al., 2019). From the great variety of capture techniques available for monitoring freshwater fish in rivers, electrofishing has been proven to most representatively determine abundance and demographic structure of fish assemblages in different habitats (Mueller et al., 2017b; Schotzko & Gassner, 2009), although the exclusive use of this technique might still lead to false assumptions on the real fish community (Erős et al., 2009). Capture methods can be applied and standardised in different ways, depending on the aims and scope of a monitoring programme. Electrofishing for instance can be conducted by fishing stretches (DeLury, 1951; Pander & Geist, 2010) of a certain length or by applying point abundance sampling (Copp, 2010). Fishing sampling stretches is the means of choice when the goal is to assess the biological integrity of a larger reach in a stream ecosystem, as it provides a rather holistic picture on the demographic and taxonomic assemblages of the fish community (Radinger et al., 2019). As shown in Chapter 9 and several other studies (see e.g. Pander & Geist, 2010; Pander et al., 2017), a multiple replicated stretch length of 30 m combined with multivariate analysis tools is well suited to determine differences in the fish community between restored

and unrestored habitats in larger reaches. In contrast, point abundance sampling allows for higher spatio-temporal resolution in fish distribution and can be modified to particularly target young and small fishes (Copp, 2010; Scholten, 2003), yet is likely to underestimate larger fish. However, the great majority of catch-dependent sampling techniques exclude fish eggs and larvae, which constitute an important criteria for a holistic fish community sampling. These life stages are particular sensitive and thus provide a good monitoring tool for the integrity of a river system and the success of conservation efforts (Schiemer et al., 2002). As the results in Chapter 8 revealed, integrating eggs and larvae in freshwater fish monitoring programmes combined with unambiguous species identification based on DNA barcoding can also detect species that would have remained undetected using classic fish monitoring methods targeting the juvenile and adult life stages only.

Most capture-depended monitoring sampling techniques come with the limitation of providing only spatio-temporal snapshots in species distribution, however, understanding of migration and distribution patterns of fish on an individual level marks an essential basis for effective conservations strategies, particularly in the context of stream restoration measures for reestablishing connectivity of aquatic habitats (Brownscombe et al., 2022; Ovidio et al., 2017). Fish marking techniques, especially active and passive telemetric techniques that do not require a recapture of fish once tagged, can provide a powerful tool for such monitoring aims as they allow a high spatio-temporal resolution and long-term observation on fish distribution on the individual level (Cooke et al., 2004; Lucas & Baras, 2000). However, such monitoring techniques have rather high technical requirements and are therefore costly, especially when applied in large-scale monitoring programmes. Telemetric techniques are also constraint to fish sizes > 50mm and thus are only applicable for juvenile and adult fish (Richard et al., 2013). However, the understanding of spatio-temporal distribution patterns of eggs and larvae is also important for the design and evaluation of habitat restoration measures (Glas et al., 2020; Lechner et al., 2014a), although being widely overlooked in fish monitoring programmes (Radinger et al., 2019). As stated above, existing mass-marking techniques for these life stages do not allow for an individual assignment (although batch marking is possible; see Lechner et al., 2014a) and previous studies revealed that recapture rates of marked larvae in the wild are very small (Lechner et al., 2014a). In addition, the very restricted availability of fish eggs and larvae in the wild, particularly of endangered or rare species, further hampers standardised distribution experiments with these life stages. In such cases, biodegradable

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surrogates can provide an effective solution to conduct standardised laboratory or field experiments throughout the year (Lechner et al., 2014a; Scherbaum et al., 2022).

This thesis clearly demonstrates that a holistic monitoring of fish communities must encompass a wide range of available tools (including classic and advancing techniques) and consider the strengths and limitations of each technique. In doing so, crucial information on the success of conservation efforts can be obtained, providing a vital basis for designing and adapting future conservation strategies.

11. Outlook

As the results of this thesis point out, combating the loss of freshwater fish biodiversity is a complex task that requires detailed knowledge of the ecology of the target fish species, including all life stages and the associated habitat requirements, as well as the strengths and pitfalls of conservation measures and methods for monitoring them. Moreover, conservation efforts need to integrate all levels of biodiversity organisation, from the genetic to the ecosystem scale, taking into account the multiple spatial and temporal dimensions of stream ecosystems.

Chapters 5 & 6 of this thesis provide important new insights into the early life ecology of *Chondrostoma nasus* through the identification of distinct intraspecific differences in egg characteristics and a systematic understanding of the susceptibility of eggs and larvae to fine sediment infiltration in spawning substrates. As it is likely that some of the observed results will also apply to other species, these results can be used as a stimulus to further investigate the ecological traits of other substrate-spawning cyprinids with a similar ecology such as *Barbus barbus*, *Squalius cephalus*, *Leuciscus leuciscus* and *Phoxinus phoxinus*.

In addition, the results of Chapters 4, 7, 8, & 9 of this thesis show that effective conservation management requires a detailed knowledge of the strengths and pitfalls of specific conservation measures, as they are often not sufficiently evaluated. This can only be achieved by using a broad range of conventional and modern monitoring techniques. Moreover, the integration of early life stages into the assessment of restoration actions may reveal additional features of restoration measures as the outcomes of Chapter 8 demonstrate. The approach developed in this study to unambiguously and inexpensively identify larval fish assemblages by combining morphometric and genetic analysis may provide a useful technique for future monitoring of ichthyoplankton communities. As it is not determined to any specific fish community, it can easily be transferred to the taxonomic analysis of fish eggs and larvae in other rivers. The integration of early life stages into fish community assessments, combined with the application of the novel identification approach, can provide important information on the recruitment success of endangered fish populations, which is critical for successfull conservation management.

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Detailed knowledge of fish species ecology, conservation tools and monitoring methods is not only important for the successful implementation of individual conservation measures at the local level, but also for decision-makers who are responsible for tailoring policies at the ecosystem scale. However, the challenge of conserving freshwater fish biodiversity should not only be seen as a scientific and political task, but also as a social process, as it needs to be widely accepted by society (Wohl et al., 2015). Furthermore, conservation efforts often rely on activities of practitioners at a local level (Baker & Eckerberg, 2013; Kondolf & Yang, 2008), which holds true for both supportive breeding and stream restoration. This was also evident in the measures evaluated in this thesis, which were implemented either through the personal motivation of angling clubs (Chapter 4 & 7) or through the legal obligations of power plant operators (Chapter 8 & 9). In light of a looming mass extinction of freshwater biodiversity, mobilising practitioners to support conservation efforts is certainly an important component (Twardek, et al., 2021). However, it is equally important that such efforts are evaluated and guided by scientific monitoring and advice. If this is not done, there is a risk that these actions will have unintended and even detrimental effects, such as those observed with the supportive breeding initiative in Chapter 4. Providing this knowledge and guidance through scientific research and translating it into recommendations for policy-makers and practitioners is one of the most important tasks for conservation scientists (Geist, 2015; Harrison et al., 2018). As this thesis has shown, this can be achieved through systematic scientific case studies and the communication of the results achieved.

Although conservation actions are often planned and implemented at a local level they need to be embedded in strong and holistic policies that aim to conserve biodiversity at the ecosystem scale. These policies should provide the framework for a comprehensive and integrative conservation management by setting goals, timelines and measures to achieve and monitor the progress of conservation actions. In contrast to the implementation of individual conservation measures, such frameworks should not focus on, and thus be limited to, certain target species, but rather aim to restore key processes in freshwater ecosystems. While individual conservation measures can be successful in supporting single aspects of biodiversity, e.g. genetic or community diversity, they cannot address the full complexity of biodiversity organisation. On the one hand, the implementation of a holistic and process-based conservation approach clearly faces greater challenges and requires a far greater commitment of resources than the sporadic implementation of individual conservation

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measures. On the other hand, it is likely to benefit the conservation of freshwater biodiversity across different taxonomic groups and over much larger and more sustained spatial and temporal scales.

12. Publication List

12.1 Publications related to this thesis

Stoeckle, B. C., Mueller, M., **Nagel, C.**, Kuehn, R., & Geist, J. (2022). A conservation genetics perspective on supportive breeding: A case study of the common nase (*Chondrostoma nasus*). Aquatic Conservation: *Marine and Freshwater Ecosystems*, *32*(10), 1596-1605.

Nagel, C., Spiessl, C., Pander, J., & Geist, J. (2021). SEM images reveal intraspecific differences in egg surface properties of common nase (*Chondrostoma nasus* L.). *Journal of Applied Ichthyology*, *37*(5), 770-778.

Nagel, C., Pander, J., Mueller, M., & Geist, J. (2020). Substrate composition determines emergence success and development of European nase larvae (*Chondrostoma nasus* L.). *Ecology of Freshwater Fish*, *29*(1), 121-131.

Nagel, C., Mueller, M., Pander, J., & Geist, J. (2020). Making up the bed: Gravel cleaning as a contribution to nase (*Chondrostoma nasus* L.) spawning and recruitment success. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(12), 2269-2283.

Nagel, C., Mueller, M., Pander, J., Stoeckle, B. C., Kuehn, R., & Geist, J. (2021). Going with the flow: Spatio-temporal drift patterns of larval fish in a large alpine river. *Freshwater Biology*, *66*(9), 1765-1781.

Pander, J., **Nagel, C.**, Ingermann, H., & Geist, J. (2022). Water level induced changes of habitat quality determine fish community composition in restored and modified riverbanks of a large alpine river. *International Review of Hydrobiology*, *107*(1-2), 46-59.

12.2 Further publications not included in this thesis

Duerregger, A., Pander, J., Palt, M., Mueller, M., **Nagel, C.**, & Geist, J. (2018). The importance of stream interstitial conditions for the early-life-stage development of the European nase (Chondrostoma nasus L.). *Ecology of Freshwater Fish*, *27*(4), 920-932.

Knott, J., **Nagel, C.** & Geist, J. (2021). Wasted effort or promising approach – Does it make sense to build an engineered spawning ground for rheophilic fish in reservoir cascades?. *Ecological Engineering*, *173*, 106434.

Meulenbroek, P., Drexler, S., **Nagel, C.**, Geistler, M., & Waidbacher, H. (2018). The importance of a constructed near-nature-like Danube fish by-pass as a lifecycle fish habitat for spawning, nurseries, growing and feeding: a long-term view with remarks on management. *Marine and Freshwater Research*, *69*(12), 1857-1869.

Nagel, C., Mizerakis, V., Pander, J., & Geist, J. (2022). The overlooked contribution of a fish bypass channel to the density and diversity of macroinvertebrate drift in a heavily modified river system. *River Research and Applications*, *38*(10), 1696-1707.

Pander, J., **Nagel, C.**, & Geist, J. (2021). Integration of constructed floodplain ponds into nature-like fish passes supports fish diversity in a heavily modified water body. *Water*, *13*(8), 1018.

Pander, J., **Nagel, C.**, & Geist, J. (2022). Effects of a Hydropower-Related Temporary Stream Dewatering on Fish Community Composition and Development: From Ecology to Policy. *Frontiers in Environmental Science*, *10*.

Pont, D., Meulenbroek, P., Bammer, V., Dejean, T., Erős, T., Jean, P., Lenhardt, M., **Nagel, C.**, Pekarik, L., Schabuss, M., Stoeckle, B. C., Stoica, E., Zornig, H., Weigand, A., & Valentini, A. (2022). Quantitative monitoring of diverse fish communities on a large scale combining eDNA metabarcoding and qPCR. *Molecular Ecology Resources*.

Scherbaum, S., **Nagel, C.**, Fuchs, Y., & Geist, J. (2022). Characterizing egg transport of *Chondrostoma nasus* (L.): a combined laboratory and field experiment. *Journal of Ecohydraulics*, 1-11.

12.3   Oral presentations

Nagel, C., Pander, J., Mueller, M., & Geist, J. (2019). Neue Erkenntnisse zur Laichökologie der Nase (*Chondrostoma nasus*). 28. Fachtagung Zoologischer und botanischer Artenschutz in Mitteleuropa. Bad Blankenburg.

Nagel, C., Mueller, M., Pander, J., Stoeckle B. C., Kuehn R., & Geist, J. (2021). Fish larvae drift in regulated large alpine rivers- a case study from the River Inn. 44th Annual Larval Fish Conference. Webex.

Nagel, C., Pander, J., & Geist, J. (2021). Das Bett bereiten- Zur Bedeutung der Substratqualität für den Laicherfolg der Nase. 18. Fachtagung Gewässerökologie und Fischartenschutz. Jena.

13. Author contributions to the chapters

Chapter 4: A conservation genetics perspective on supportive breeding: A case study of the common nase (*Chondrostoma nasus*)

Conceptualisation: CN, BS, MM and JG; methodology: BS, CN, MM, RK and JG; validation: RK and JG; formal analysis: BS; investigation: CN, BS and MM; resources: RK & JG; writing—original draft preparation: BS, CN, MM and JG; writing—review and editing: BS, CN, MM, RK and JG

Chapter 5: SEM images reveal intraspecific differences in egg surface properties of common nase (*Chondrostoma nasus* L.)

Conceptualisation: CN and CS; methodology: CN and CS; validation: JP and JG; formal analysis: CN and CS; investigation: CN and CS; resources: JG; writing—original draft preparation: CN; writing—review and editing: CN, CS, JP and JG

Chapter 6: Substrate composition determines emergence success and development of European nase larvae (*Chondrostoma nasus* L.)

Conceptualisation: CN, MM, JP and JG; methodology: CN, MM, JP and JG; validation: JG; formal analysis: CN; investigation: CN; resources: JG; writing—original draft preparation: CN; writing—review and editing: CN, JP, MM and JG

Chapter 7: Making up the bed: Gravel cleaning as a contribution to nase (*Chondrostoma nasus* L.) spawning and recruitment success

Conceptualisation: CN; methodology: CN, MM, JP; validation: JG; formal analysis: CN; investigation: CN; resources: JG; writing—original draft preparation: CN; writing—review and editing: CN, MM, JP and JG

Chapter 8: Going with the flow: Spatio-temporal drift patterns of larval fish in a large alpine river

Conceptualisation: CN, MM, JP and JG; methodology: CN, BS; validation: JG; formal analysis: CN; investigation: CN; resources: RK & JG; writing—original draft preparation: CN; writing—review and editing: CN, MM, JP, BS, RK and JG

Chapter 9: Water level induced changes of habitat quality determine fish community composition in restored and modified riverbanks of a large alpine river

Conceptualisation: JP and CN; methodology: JP, CN and HI; validation: JG; formal analysis: JP and CN; investigation: CN and JP; resources: JG; writing—original draft preparation: JP, CN and HI; writing—review and editing: JP, CN, HI and JG

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15. Supporting Information

Supporting information 1: Sampling date and total count (n) of drifting larvae with absolute (n) and relative (%) proportions of subsamples used for assessing the genetic diversity of the wild offspring in the River Mangfall and the River Sims

	Date	Larvae total (n)	Larvae subsample (n)	Larvae subsample (%)
Mangfall	April 19	40	6	15%
	April 20	22	3	14%
	April 21	40	6	15%
	April 22	26	4	15%
	April 24	281	29	10%
Sims	April 21	20	6	30%
	April 22	27	9	33%
	April 23	61	18	30%
	April 24	20	6	30%
	April 25	29	9	31%

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Supporting information 2: Results of LMM testing for the effect of treatment and incubation time on larvae length (Model 1)

Contrast	Z	Mean difference (± SE)
A - R	5.48	1.40 ± 0.25 p < 0.001
B - R	4.55	1.15 ± 0.25 p < 0.001
C - R	3.47	0.87 ± 0.25 p < 0.01
D - R	3.01	0.76 ± 0.25 p < 0.05
E - R	4.83	1.22 ± 0.25 p < 0.001
F - R	2.67	0.67 ± 0.25 p = 0.12
B - A	-0.84	-0.25 ± 0.29 p = 1.00
C - A	-1.81	-0.53 ± 0.29 p = 0.84
D - A	-2.15	-0.63 ± 0.29 p = 0.44
E - A	-0.59	-0.17 ± 0.29 p = 1.00
F - A	-2.52	-0.73 ± 0.29 p = 0.18
C - B	-0.96	-0.28 ± 0.29 p = 1.00
D - B	-1.31	-0.39 ± 0.29 p = 1.00
E - B	0.25	0.07 ± 0.29 p = 1.00
F - B	-1.66	-0.48 ± 0.29 p = 1.00
D - C	-0.36	-0.11 ± 0.29 p = 1.00
E - C	1.21	0.35 ± 0.29 p = 1.00

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F - C	-0.70	-0.20 ± 0.29 p = 1.00
E - D	1.56	0.46 ± 0.29 p = 1.00
F - D	-0.33	-0.01 ± 0.29 p = 1.00
F - E	-1.91	-0.56 ± 0.29 p = 0.72

Notes: Model= length ~ treatment + (1 | compartment)

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Supporting information 3: Overview on aims, methods, data basis and the respective statistical testing

Study aim		Method	Replicates	Statistical testing	Displayed in
(i)	Fine sediment content	Freeze-core	3 (per time, treatment and river)	descriptive	Figure 26
(ii)	Spawning site use	Visual counts of spawners	1 (per treatment and river)	descriptive	Text
		Spawning boxes	6 (per treatment and river)	Kruskal-Wallis	Figure 27
(iii)	Infiltration of eggs and larvae into the interstitial	Freeze-core	Eggs: 3 (per treatment and river) Larvae: 3 (per treatment and river)	descriptive	Table 10 & 11
(iv)	Development and timing of emergence	Drift net sampling	Eggs: 11 (per treatment and river) Larvae: 13 (per treatment and river)	Linear Mixed Models	Figure 28
		Measuring larvae size	13 (per treatment and river, comprising 1,699 larvae)	Linear Mixed Models	Figure 29

Supporting information 4: Absolut (n) and relative (%) distribution of all species and families caught separated for each life stage. Adult and juvenile fish refer to individuals caught via electrofishing, larvae and eggs to individuals caught via drift sampling

Species	Adult and juvenile		Drifting larvae		Drifting eggs	
	n	%	n	%	n	%
Cottidae	368	30.9	227	10.9		
Cottus gobio	368	30.9	227	10.9		
Cyprinidae	622	52.2	648	33.3	33	11.7
Abramis brama	10	0.8	84	4.0	3	1.1
Alburnoides bipunctatus	1	0.1				
Alburnus alburnus	32	2.7	9	0.4		
Aspius aspius			16	0.8		
Barbatula barbatula	110	9.2				
Barbus barbus	72	6.0	100	4.8		
Chondrostoma nasus	75	6.3	289	13.9	14	4.9
Cyprinus carpio	4	0.3				
Gobio gobio	3	0.3	1	0.0		
Leuciscus idus			2	0.1		
Leuciscus leuciscus	58	4.9				
Phoxinus phoxinus	1	0.1				
Pseudorasbora parva	78	6.5	1	0.0		
Rhodeus amarus	1	0.1				
Rutilus rutilus	17	1.4	1	0.0		
Scardinius erythrophthalmus	2	0.2				
Squalius cephalus	155	13.0	145	7.0	16	5.7
Tinca tinca	3	0.3				
Esocidae	3	0.3				
Esox lucius	3	0.3				
Gadidae	1	0.1				
Lota lota	1	0.1				
Gasterosteidae	150	12.6	29	1.5		
Gasterosteus aculeatus	150	12.6	29	1.4		
Percidae	7	0.6	44	2.2		
Perca fluviatilis	7	0.6	42	2.0		
Sander lucioperca			2	0.1		
Petromyzontidae	3	0.3				
Eudontomyzon spec.	3	0.3				
Salmonidae	38	3.2	66	3.2		
Coregonus spec.			2	0.1		
Hucho hucho	2	0.2				

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Oncorhynchus mykiss	7	0.6				
Salmo trutta fario	4	0.3				
Thymallus thymallus	25	2.1	64	3.1		
n.d.			55	2.6	250	88.3
∑	1,192		1,069		283	

Supporting information 5: Abiotic and structural habitat parameters at the three habitat types CP = concrete profile, RR = restored riverbank in % coverage, BR = bank riprap in % coverage. EC = electric conductance standardised to 20°C in micro Siemens per centimetre, pH = pH value, O_2 = oxygen concentration in milligrams per litre [mgL⁻¹] , CSS = current speed 10 cm below surface in meters per second [ms⁻¹], CSB = current speed 10 cm above substratum in meters per second [ms⁻¹], T = temperature in degrees Celsius [°C], Turb = turbidity as nephelometric turbidity unit [NTU], Depth = water depth in centimetres [cm]. C = concrete in % coverage, B = boulder in % coverage, G = gravel in % coverage, S = sand in % coverage, CC = canopy cover in % coverage, DW = deadwood in % coverage, VEG = bank vegetation in % coverage, R = roots in % coverage. Values are given as mean ± standard deviation [minimum to maximum]

S	EC [μS cm⁻¹]	O$_2$ [mg L⁻¹]	T [°C]	Turb [NTU]	pH	Depth [cm]	CSS [m s⁻¹]	CSB [m s⁻¹]	CC [%]	VEG [%]	R [%]	DW [%]	C [%]	B [%]	G [%]	S [%]
CP																
Summer	213 ± 19	9.8 ± 0.3	14.3 ± 0.7	111.1 ± 36.3	8.1 ± 0.1	170.1 ± 44.5	0.4 ± 0.2	0.2 ± 0.1	9 ± 14	32 ± 37	1 ± 3	2.8 ± 4.2	83 ± 24	0 ± 0	0 ± 0	17 ± 24
	[191 - 238]	[8.6 - 10.1]	[13.4 - 15.1]	[64.7 - 180.4]	[8.0 - 8.3]	[112.0 - 240.3]	[0.1 - 0.6]	[0.0 - 0.4]	[0 - 50]	[0 - 85]	[0 - 10]	[0.0 - 10.0]	[50 - 100]	[0 - 0]	[0 - 0]	[0 - 50]
Autumn	333 ± 23	10.4 ± 0.2	10.3 ± 0.4	13.4 ± 6.8	8.1 ± 0.0	80.6 ± 13.0	0.4 ± 0.2	0.1 ± 0.1	7 ± 10	0 ± 0	0 ± 0	2.1 ± 4.2	34 ± 13	16 ± 10	4.7 ± 13.3	46 ± 24
	[304 - 363]	[9.9 - 10.6]	[9.8 - 10.9]	[64.7 - 180.4]	[8.0 - 8.2]	[66.7 - 110.7]	[0.1 - 0.6]	[0.0 - 0.2]	[0 - 35]	[0 - 0]	[0 - 0]	[0.0 - 15.0]	[15 - 60]	[0 - 0]	[0 - 50]	[0 - 80]
BR																
Summer	215 ± 15	9.5 ± 0.5	14.8 ± 0.7	130.3 ± 41.0	8.1 ± 0.1	203.4 ± 52.0	0.3 ± 0.1	0.1 ± 0.1	26 ± 24	77 ± 22	1 ± 2	3.9 ± 6.8	0 ± 0	69 ± 24	0 ± 0	31 ± 24
	[197 - 235]	[7.6 - 9.7]	[13.8 - 16.0]	[72.5 - 189.8]	[8.2 - 7.6]	[124.7 - 281.0]	[0.1 - 0.5]	[0.0 - 0.3]	[35 - 80]	[20 - 90]	[0 - 5]	[0.0 - 20.0]	[0 - 0]	[50 - 100]	[0 - 0]	[0 - 50]
Autumn	341 ± 34	10.4 ± 0.1	10.2 ± 0.6	13.3 ± 5.1	8.1 ± 0.0	116.6 ± 15.7	0.2 ± 0.1	0.0 ± 0.0	26 ± 24	0 ± 0	0 ± 0	0 ± 0	0 ± 0	65 ± 16	0 ± 0	35 ± 16
	[308 - 454]	[10.3 - 10.6]	[9.4 - 11.1]	[6.8 - 24.5]	[8.0 - 8.2]	[85.7 - 145.0]	[0.0 - 0.4]	[0.0 - 0.1]	[0 - 80]	[0 - 0]	[0 - 0]	[0 - 0]	[0 - 0]	[50 - 90]	[0 - 0]	[10 - 50]
RR																
Summer	211 ± 18	9.6 ± 0.2	14.4 ± 0.6	122.2 ± 50.0	8.1 ± 0.1	172.7 ± 42.3	0.3 ± 0.2	0.2 ± 0.1	1 ± 2	7 ± 10	0 ± 0	3.8 ± 7.4	0 ± 0	7 ± 15	58 ± 38	34 ± 38
	[170 - 236]	[9.1 - 10.0]	[13.5 - 15.2]	[75.9 - 211.1]	[8.0 - 8.3]	[106.7 - 283.3]	[0.1 - 0.6]	[0.0 - 0.5]	[0 - 10]	[0 - 30]	[0 - 0]	[0.0 - 20.0]	[0 - 0]	[0 - 50]	[0 - 100]	[0 - 95]
Autumn	336 ± 22	10.4 ± 0.1	10.3 ± 0.5	9.9 ± 2.9	8.1 ± 0.1	82.3 ± 18.0	0.2 ± 0.1	0.1 ± 0.1	1 ± 3	0 ± 0	0 ± 0	3.1 ± 4.8	0 ± 0	23 ± 15	24 ± 11	53 ± 19
	[304 - 364]	[10.3 - 10.5]	[9.6 - 11.0]	[6.9 - 17.2]	[8.0 - 8.3]	[36.0 - 116.3]	[0.1 - 0.6]	[0.0 - 0.4]	[0 - 10]	[0 - 0]	[0 - 0]	[0.0 - 15.0]	[0 - 0]	[0 - 50]	[10 - 60]	[20 - 90]

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Supporting information 6: List of all species caught listed in scientific and common names, their affiliation to ecological flow guilds (based on the classification of Zauner & Eberstaller, 1999) and conservation status (according to Annex II or V of the Habitats Directive, Council of the European Communities (1992), Red List (RL) Bavaria and Germany (Bohl et al. 2003). Categories: 1 = threatened with extinction, 2 = highly endangered, 3 = endangered

Scientific name	Common name	Flow guild	Conservation status		
			EU-FFH	RL-Germany	RL-Bavaria
Abramis brama	common bream	indifferent			
Alburnoides bipunctatus	spirlin	rheophil		2	2
Alburnus alburnus	bleak	indifferent			
Anguilla anguilla	European eel	indifferent		3	3
Barbatula barbatula	stone loach	rheophil		3	
Barbus barbus	European barbel	rheophil	V	2	3
Blicca bjoerkna	white bream	indifferent			
Chondrostoma nasus	common nase	rheophil		2	2
Cottus gobio	bullhead goby	rheophil	II	2	
Cyprinus carpio	carp	indifferent		3	2
Esox lucius	pike	indifferent			
Gasterosteus aculeatus	three-spined stickleback	indifferent			
Gobio gobio	gudgeon	rheophil			
Gymnocephalus cernua	ruffe	oligorheophil			
Hucho hucho	huchen	rheophil	II, V	3	1
Petromyzontidae	northern lampreys	rheophil	(II)		
Leuciscus leuciscus	dace	rheophil		3	
Lota lota	burbot	rheophil		2	2
Oncorhynchus mykiss	rainbow trout	rheophil			
Perca fluviatilis	perch	indifferent			
Phoxinus phoxinus	common minnow	rheophil		3	3
Pseudorasbora parva	stone moroko	indifferent			
Rhodeus amarus	bitterling	indifferent	II	2	2
Rutilus rutilus	roach	indifferent			

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Salmo trutta fario	brown trout	rheophil		3	
Sander lucioperca	Pike-perch	indifferent			
Scardinius erythrophthalmus	rudd	stagnophil			
Squalius cephalus	chub	indifferent			
Telestes souffia	vairone	rheophil	II	1	1
Thymallus thymallus	European grayling	rheophil	V	3	2