Water Air Soil Pollut (2023) 234:623
https://doi.org/10.1007/s11270-023-06635-8

Massive Fish Kill After the Discharge of Artificial Fertilizer into a Species Rich River in Southwestern Germany: a Conservation Case Study

Julia Gaye-Siessegger · Mark Schumann · Jan Baer · Samuel Roch · Lukas Ittner · Alexander Brinker

Received: 13 March 2023 / Accepted: 8 September 2023 / Published online: 21 September 2023

Abstract In the summer of 2015, large amounts of artificial fertilizer containing ammonium nitrate were accidentally discharged into the Jagst River along with water used to extinguish a serious fire. The incident caused a massive fish kill the first 25 km downstream of the spill and impacted fish density along a 50 km stretch of this sensitive and important river. In this study, the long-term effects of the accident on the local fish fauna were investigated, and the implemented restoration measures were evaluated. A majority of fish surviving the immediate effects of the incident exhibited massive gill damage and weakness to infections shortly after the accident, but survival over the following winter appeared unimpaired. Two years after the accident, most survived fish appeared healthy. In 2016, about 9500 individuals (500 kg fish of 11 autochthonous species) were caught in unaffected sections of the river and distributed systematically into severely affected sections. Two control sections were left unstocked. Species diversity and fish density remained low over the first winter 2015/16, but increased in autumn 2016, most likely as a result of systematic stocking measures taken in response to the disaster. Stocking and natural migration were able to restore species diversity back to pre-accident levels in due time, i.e., 36 months, but density remains lower and shows no sign of further recovery. A positive consequence of the catastrophe has been the enactment in the ensuing years of various measures to improve the resilience of the Jagst River. However, connectivity is still lacking in relevant sections of the river and this, in combination with high predation from an increasing population of cormorants, has hampered the recovery of fish stocks. Generalizable conservation measures to mitigate the impact of similar catastrophes are developed and discussed.

Keywords Ammonia · Conservation measures · Fish health · Jagst River · Recovery · Stocking

1 Introduction

Fish kills are unfortunately not uncommon in aquatic ecosystems, but detailed information pertaining to their frequency, cause and extent and the efficacy of mitigation measures is regrettably scarce. Recent data

---

Julia Gaye-Siessegger and Mark Schumann contributed equally to this work.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11270-023-06635-8.

J. Gaye-Siessegger (✉) · M. Schumann · J. Baer ·
S. Roch · A. Brinker
Fisheries Research Station Baden-Württemberg, LAZBW,
88085 Langenargen, Germany
e-mail: Julia.Gaye-Siessegger@lazbw.bwl.de

L. Ittner
Bavarian Environment Agency, 82407 Wielenbach,
Germany

A. Brinker
University of Konstanz, 78457 Konstanz, Germany

from Switzerland suggests that small-scale sudden mass mortalities occurred on average every second day following anthropogenic impacts in the period from 1990 to 2018, with more than 90% resulting from discharges of agricultural, municipal sewage, or industrial effluents (BAFU, 2020). A recent incident affecting several hundred kilometers of the Oder River in Poland and Germany in August 2022 reveals the challenges inherent in identifying causes and mitigations for mass fish kills as well as the rarity of scientific data and analyses (IGB, 2022). After weeks of intensive investigation, authorities assumed that the mortality was caused by a combination of industrial waste disposal and climate change effects. High salt loads in combination with increasing temperature led to a massive spread of the toxin-producing golden-brown algae (*Prymnesium parvum*) and the subsequent death of hundreds of tons of fish as well as other animal species, e.g., mussels and snails (IGB, 2022). In the present case of the Jagst River in 2015, the massive kill was triggered by the accidental introduction of nitrogen in one of the most fish-rich and ecologically important rivers in southwestern Germany (Chucholl et al., 2019).

Toxic levels of nitrogen, usually involving ammonia, are often implicated in fish kills (BAFU, 2020; Francis-Floyd et al., 2009; La & Cooke, 2011). In water, non-ionized ammonia (NH_3) occurs in an equilibrium with ionized ammonium (NH_4^+) (Francis-Floyd et al., 2009; Levit, 2010), of which the former is toxic to fish and other aquatic organisms (Randall & Tsui, 2002; U.S. EPA, 2013). The equilibrium is affected by both pH and water temperature. The ratio of NH_3 to NH_4^+ increases strongly with increasing pH, while elevated temperatures cause a more moderate shift towards NH_3 (U.S. EPA, 2013; Levit, 2010). Ammonia is ubiquitous in aquatic environments as an intermediate product of the natural nitrogen cycle, produced by microorganisms involved in the decomposition of organic matter (Randall & Tsui, 2002). It is also a product of fish metabolism, excreted via the gills (U.S. EPA, 2013). The process of microbial oxidation of ammonium to nitrate produces nitrite, which is also highly toxic to fish and other aquatic organisms (U.S. EPA, 2013). The same degradation processes can also have a noticeable effect on the oxygen balance of a water body, and high NH_3 levels can inhibit fish respiration in several ways. Elevated NH_3 in the water can reduce or even reverse the gradient for diffusion from the gills and thereby lead to accumulation inside the fish, by impaired excretion or uptake of ammonia (Randall & Tsui, 2002). Fish mortality occurs when plasma levels of ammonia begin to exert a variety of chemical effects on cellular processes including neurotoxic impacts (Ip & Chew, 2010). Exposure to ammonia can cause histopathological changes in the gill epithelium, which may lead to sublethal or even lethal effects. In general, fish are more sensitive to elevated ammonia levels than invertebrates (Arthur et al., 1987).

A striking example of ammonia contamination occurred in summer 2015 in southwestern Germany. On the night of 22 to 23 August, after a fire broke out in a mill at Lobenhausen/Kirchberg, large amounts of artificial fertilizer containing ammonium nitrate were washed into the Jagst River along with water used to extinguish the blaze (LUBW, LAZBW, and Regierungspräsidium Stuttgart, 2016; LUBW, LAZBW, and Regierungspräsidium Stuttgart, 2017). The spillage took place at km 118.5 of the Jagst River, a lowland section subject to a variety of anthropogenic impacts (weirs, straightening, shoreline stabilization, etc.) but nevertheless highly biodiverse, with a fish community dominated by cyprinid species. The district office estimated that the water used to fight the fire carried approximately 1.3 tons of total ammonia nitrogen (TAN) into the river. Besides TAN, apparently, no other toxic substances or chemicals were released. Flow rates were low at the time due to an extended dry period (with discharges of 1.8 m^3/s at Lobenhausen and 6.0 m^3/s at the river mouth compared to mean rates of 10.0 m^3/s and 19.0 m^3/s, respectively) (LUBW, LAZBW, and Regierungspräsidium Stuttgart, 2017). This limited the potential for dilution of the harmful pollutant and relatively slow passage of the pollution wave downstream. Nevertheless, during the first 30–50 km downstream of the incident, the high initial mortality rates decreased strongly due to a continuous dilution and peak reduction of the TAN wave and with it the ammonia concentration (LUBW, LAZBW, and Regierungspräsidium Stuttgart, 2016; Fig. 1). However, even 70 km downstream of the point of discharge, peak NH_3-N values still exceeded LC 50 concentrations for fish such as brown trout (*Salmo trutta*) and common carp (*Cyprinus carpio*) for which acute toxicity occurs at 0.43 mg L^{-1} and 0.44 mg L^{-1} NH_3-N, respectively (U.S. EPA, 2013).

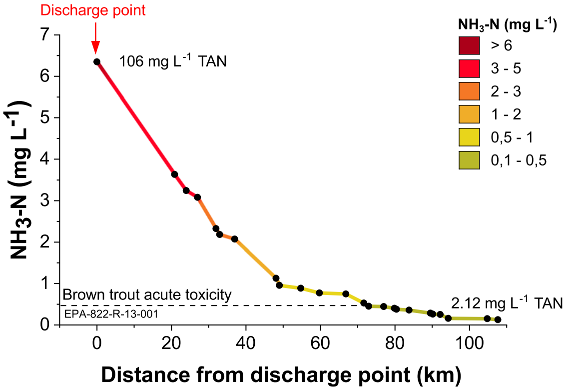

Fig. 1 Development of maximum ammonia (NH_3-N) concentrations in the Jagst River after a large spill of artificial fertilizer containing ammonium nitrate following progress of the pollution wave (from day one until day five after the incident). Ammonia levels were calculated from total ammonia nitrogen (TAN), pH, and water temperature measured in the river (Emerson et al., 1995; LUBW, LAZBW, and Regierungspräsidium Stuttgart, 2016). The dashed line marks the acute toxicity values for a representative fish species (U.S. EPA, 2013)

In the first 25 km below the spill, almost all fish died. Direct mortality then decreased slightly over the following 25 km, although the pollutant levels still exceeded concentrations reported to be lethal to a majority of native fish species. About 20 tons of dead fish, mainly cyprinids such as barbel (*Barbus barbus)*, roach (*Rutilus rutilus)*, chub (*Squalius cephalus*), and bream (*Abramis brama*), were collected and taken to carcass disposal. Presumably, a further unknown quantity of dead fish remained in the river. As an immediate action, where possible, electric fishing was used to catch as many fish as possible (given limitations of manpower, gear, and time) from downstream sections before the polluted water arrived and transferred to unaffected sections (including tributaries, isolated secondary channels, bypasses). Furthermore, massive aeration measures were initiated by local administrative authorities and continued day and night for several days after the accident, although there was supersaturation during the day. From about 15 km below the spill, attempts were made to divert polluted water through bypassing mill channels to protect the fish communities in the main river. However, despite all these measures, fishing clubs, landowners, and nature protection agencies reported low or non-existent fish stocks in the aftermath of the accident. After a few months, the "Jagst River Program" was launched by environmental and agricultural authorities with the aim of reviving and improving the ecology of damaged sections of the river in the long term (https://um.baden-wuerttemberg.de/de/umwelt-natur/wasser-und-boden/jagst/). In addition, a science-based stocking program was initiated to swiftly restore ecological balance to local fish communities and boost natural reproductive potential. However, due to the high density of piscivorous birds in this area, the efficacy of stocking was questioned in advance by the local fishing clubs.

The aim of the present study was (i) to document the consequences of the pollution incident on the fish population in the Jagst River, (ii) to assess the efficacy of stocking measures compared to natural recolonization, (iii) to investigate the importance of habitat connectivity, (iv) to discuss the potential impact of increasing pressure from piscivorous birds on the vulnerable fish community, and (v) finally to appraise implemented measures and consider other which may improve the future resilience of the Jagst River.

2 Methods

2.1 Study Area

The Jagst River is a 190 km long tributary in the Neckar/Rhine system in southwestern Germany. Land use in the 1825 km^2 catchment area (Fig. 2) comprises 64.8% agriculture, 29.7% forest, 5.0% urban areas, and 0.5% other uses (Regierungspräsidium Stuttgart, 2021). In the upper part of the study area (about 93 km), the river is dominated by rhithral habitats, which previously provided stronghold areas for a number of rheophilic, often endangered or protected species including bullhead (*Cottus gobio*), spirlin (*Alburnoides bipunctatus*), common nase (*Chondrostoma nasus*), and barbel. The lower part of the river is potamal in character, supporting higher percentages of generalist species like perch (*Perca fluviatilis*) and roach (about 25 km). In the affected section, the river has a mean wetted width of 18 m and a mean water depth of 1.5 m. Mean discharges at Lobenhausen are 10 m^3/s, increasing to 19 m^3/s close to the confluence with the Neckar River. Historically, the Jagst River featured runs, riffles, and fast-flowing deeper pools with coarse substrata, alternating with slow-flowing parts where the substrata were typically

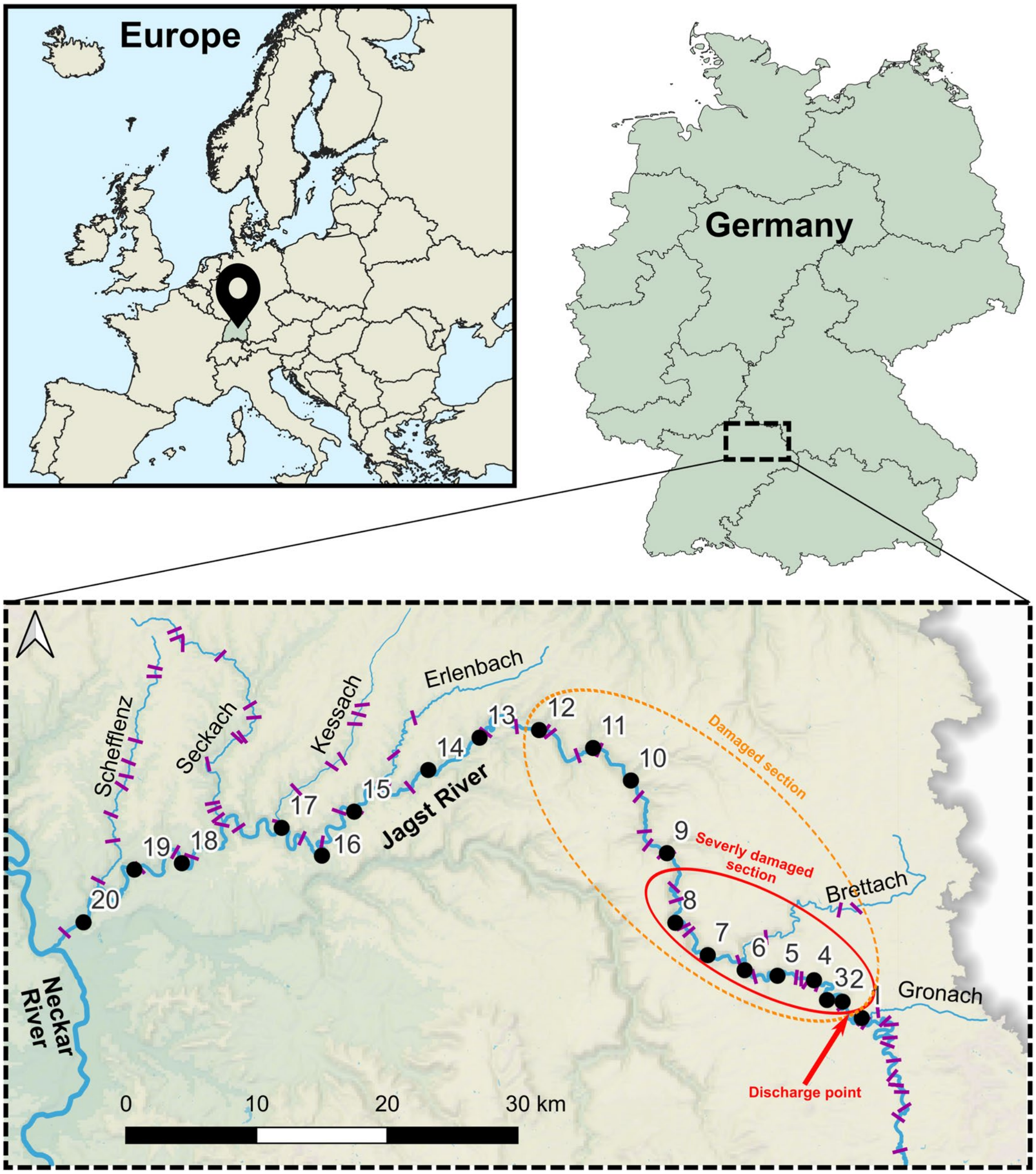

Fig. 2 Course of the Jagst River, a tributary of the Neckar River in southwestern Germany. Orange ellipse indicates the section affected by fertilizer contamination in 2015, and red ellipse shows the section where the impact was most severe; numbered black dots indicate sampling locations, and purple dashes transverse structures

sandy or muddy. However, the construction of 45 hydropower stations over the last 100 years has led to considerable fragmentation of the watercourse as well as habitat alteration and declining water quality. The chemical status of the damaged section of the Jagst River failed to achieve good, because the concentrations of endosulfan, bifenox, brominated diphenyl ethers, and mercury exceed the relevant Environmental Quality Standards (Regierungspräsidium Stuttgart, 2021). However, no fish kills associated with these chemicals have been observed to date in this river section.

2.2 Fish Monitoring

To assess the status of the fish stock in the wake of the incident, an extensive fishing campaign was initiated on September 1, 2015, 2 weeks after the mass kill event. Between autumn 2015 and 2018, twenty stretches were surveyed biannually in spring and autumn (Fig. 2). From 2019 on, this was reduced to annual monitoring on selected stretches. Sampling was conducted using a single anode, 600 V DC electrofishing setup (8 kW, EFKO Elektrofischfanggeräte GmbH). All captured fish were identified to species, allocated to a 5 cm length class, counted, and released. The catch per unit effort (CPUE) was calculated as caught individuals per 100 m river stretch, hereafter referred to as fish density. To compare the new monitoring results with those from years preceding the incident, data from electric fishing surveys of the same sampling stretches between 2005 and 2013 (held in the online database of the Fisheries Research Station, Table S1) were summarized. Using this historical data, it was possible to calculate a mean fish density for each sampling location prior to the fish kill. In two severely damaged sections of the main river, natural immigration of fish from unaffected sections was possible: in section 2 fish from upstream of the discharge point, and in section 6 from a tributary, the Brettach (Fig. 2).

To monitor the effect of attempts to direct the flow of polluted water through bypassing mill channels to protect fish communities in the main river, in October 2015 (7 weeks after the accident), electric surveys were conducted in 10 mill channels and in 10 corresponding sections of the main river. All stretches were located between 15 and 33 km downstream of the discharge point. Monitoring was also carried out in seven further mill channels and corresponding main river sections where the polluted water was not bypassed, located between 1 and 14 km downstream of the discharge point.

2.3 Fish Health

Gill condition was used as a proxy for fish health after the incident in the Jagst River. Gill tissues are regarded as the most exposed and pollution-sensitive of fish tissues, with numerous pollutants shown to cause histopathological changes (Poleksić & Mitrović-Tutundžić, 1994). Chub and gudgeon (*Gobio gobio*) were collected randomly during the biannual electric fishing surveys between autumn 2015 and autumn 2017 as representatives of lotic and benthic fish stocks and examined for signs of gill damage. To avoid sampling of stocked individuals from autumn 2016 on, fish were only taken if their size was significantly above or below stocking size or if they were caught from the two unstocked sections of the river. All fish were euthanized with an overdose of clove oil (1 mL L^{-1}) and a gill cut according to the German Animal Welfare Act ("Tierschutzgesetz"). All fish were measured to the nearest millimeter total length (TL) and weighed wet to the nearest gram, shortly after capture, then transported on ice to the lab for immediate microscopic inspection (Stemi SV6, Zeiss, Oberkochen, Germany) of the gill.

Alterations to gill tissues were assessed according to the modified organ index I_{org} described by Bernet et al. (1999). Gills were excised and examined for macro-pathological alterations, including anemia, proliferation, and necrosis, and for mucus occurrence. Briefly, the alterations were classified according to the intensity and distribution of damage, with an importance factor (w) attributed to each alteration (anemia 1, mucus occurrence 1, proliferation 2, and necrosis 3). Table S2 presents the criteria used to assign a score value for each alteration. From these ratings, the gill damage index (I_{GD}) was calculated as

$$I_{GD} = \sum_{df} \left(a_{df} * w_{df} \right),$$

where *df* is the diagnostic finding, *a* is the score value, and *w* is the importance factor. The index represents the degree of gill damage, with higher values representing more severe pathological alterations.

2.4 Stocking

In 2016, a stocking plan was developed based on the baseline data for the most severely damaged section of the Jagst River representing the pre-existing fish community in an unimpaired state (Table 1 + S3) (Dussling, 2009). This reference community had been determined as part of the implementation of the Water Framework Directive (WFD) (European Community, 2000). The main species chosen for future stocking were those which dominated this baseline community, namely, minnow (*Phoxinus phoxinus*), stone loach (*Barbatula barbatula*), barbel, chub, dace

Table 1 Stocked species, their number (Ind), and masses (Mass) in 2016, 2017, and 2018. The last two columns give the goal quantities for stocking based on baseline reference data and actual quantities achieved (as percentages of previous population estimate). Protected and endangered species are given in bold

Species	2016		2017		2018		Total		Reference	
	Ind (*n*)	Mass (kg)	Ind (*n*)	Mass (kg)	Ind (*n*)	Mass (kg)	Ind (*n*)	Mass (kg)	Goal (%)	Actual value (%)
Barbel (*Barbus barbus*)	875	124.1	112	10.1	1	0.04	988	125.5	10.6	7.0
Bleak (*Alburnus alburnus*)	296	9.2	3	0.1	0	0.0	299	9.3	2.5	2.1
Bullhead (*Cottus gobio*)	1175	8.8	560	2.1	365	1.2	2100	9.6	8.1	15.0
Chub (*Squalius cephalus*)	710	185.5	229	13.6	10	0.8	949	195.2	10.6	6.8
Common nase (*Chondrostoma nasus*)	123	66.9	16	11.5	73	86.7	212	165.1	5.8	1.5
Dace (*Leuciscus leuciscus*)	347	14.8	27	2.2	1	0.2	375	17.1	10.6	2.7
Gudgeon (*Gobio gobio*)	532	11.9	35	0.5	12	0.1	579	12.0	10.6	4.1
Minnow (*Phoxinus phoxinus*)	2122	15.1	1429	7.8	441	1.6	3992	16.0	13.4	28.5
Roach (*Rutilus rutilus*)	400	18.0	30	0.5	0	0.0	430	18.5	3.8	3.1
Spirlin (*Alburnoides bipunctatus*)	2005	27.5	761	4.9	14	0.1	2780	28.1	10.6	19.8
Stone loach (*Barbatula barbatula*)	903	9.6	161	1.7	263	6.3	1327	10.2	13.4	9.5
Total	9488	491.4	3363	55.1	1180	97.0	14,031	643.5		

(*Leuciscus leuciscus*), gudgeon, spirlin, bullhead, and common nase (predators explicitly excluded; Table S3A). Stocking fish of foreign provenance was explicitly excluded in order to maintain local genetic integrity (Antognazza et al., 2016). The goal was to stock the 25 km long (45 ha) severely damaged section with around 10% of the standing stock prior to the incident (roughly 200 kg/ha, based on back calculations from earlier electric fishing surveys) to serve as a basis for natural reproduction. Achieving this would require a catch of around 27,000 local fish of eleven different species with a total mass of around 910 kg (20 kg/ha). In July and September 2016, fish were caught in different unaffected sections and tributaries (Fig. S1) using the same electric fishing device deployed for fish monitoring, placed in aerated fish transport tanks (volume: 1–2 m^3), and transported directly (duration: 25–120 minutes) to selected sections in the severely damaged section of the river (Fig. S1). Fish of different length classes (juvenile and adult fish, Table S3B) were used for stocking purposes. After acclimatization to ambient water temperatures, they were released uniformly across the whole river section. Two sections were excluded from the stocking measures, one directly below the discharge point at Lobenhausen (Fig. 2, sampling section 2, 1.5 km long) and the other about 10 km downstream at Diembot (Fig. 2, sampling section 5, 3 km long). The latter is isolated by insurmountable weirs (Fig. S1). Due to turbid water conditions, low capture rates, and catch restrictions of the local fishing clubs for certain species, only about 50% of the target stocking numbers could be caught in 2016 (Table 1), resulting in a mean stocking density of around 10 kg/ha. In 2017 and 2018, an allowance was made for additional catches in some donor sections, thus additional stocking effort could be made for some species (Table 1). Furthermore, an agreement was established with fishing clubs and landowners along the Jagst River that no further stocking would be conducted during the subsequent monitoring period.

2.5 Bird Predation

In the winter before the accident, alone more than 150 cormorants (*Phalacrocorax carbo sinensis*) had their night roosts at the Jagst River near to the polluted river section. To appraise the impact of predation by cormorants on natural (immigration) and artificially enhanced (stocked) recovery of the fish community in the aftermath of the pollution incident, the number of cormorants at night roosts was counted during the study period at 4 weeks intervals by conservationists and anglers. Based on these records, the number of "cormorant days," i.e., days with feeding cormorants on a given section, was calculated. Pre-incident records were also available made from fishing clubs at various points along the Jagst River since 2006.

In the period August 2008 to March 2011, a derogation for lethal control of cormorants during autumn and winter was granted due to the increasing number of cormorants at the Jagst River (from around the lower half of the severely damaged section down to the Jagst-Neckar confluence, with the exception of nature reserves and surrounding buffer zones). In 2016, a derogation for lethal control was granted for the same area and time of the year but with a set maximum number of cormorants to be culled (170 individuals in 5 years and not more than 50 individuals per year) in order to rule out possible impairment of protected species (Fig. S2a and b). This derogation was justified primarily as a means to enhance fish stock recovery by minimizing bird predation. From 2018 to 2021, additional annual derogations were granted for night roosts upstream of the severely damaged area and in some nature reserves and buffer zones (Fig. S2C and D). To monitor the deterrent effect of control measures on other birds (e.g., displacement due to hunting activity), all measures were accompanied by monitoring of protected bird species since 2016 (detailed information Supplement Fig. S2).

2.6 Data Analysis and Statistics

In terms of fish health, temporal trends in I_{GD} after the accident were analyzed using a linear regression. Ordinal logistic regression assuming a Poisson distribution was used to analyze the relationship between gill condition and time after the pollution event.

In order to compare species diversity (number of species) and CPUE (number of individuals per 100 m river stretch) before and after the accident, data was first tested for homoscedasticity (Levene-Test; Levene, 1960) before choosing a parametric (Dunnet's test with control; Dunnett, 1955) or non-parametric (Dunn's test with control for joint ranks; Dunn, 1964) test. Data gathered before the incident (2005–2013) were used as a control. Temporal trends in species diversity and CPUE after the accident were investigated using general linear mixed models (GLMMs) following the general formula (Sachs, 2004):

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + \varepsilon_{ijk},$$

where Y_{ijk} is the number of species or CPUE, μ is the overall mean, α_i denotes whether stocking had been carried out (stocking yes/no), β_j denotes whether the investigated sampling site has natural immigration (immigration yes/no), γ_k is months passed after the accident (time), $(\alpha\gamma)_{ik}$ is the interaction of stocking and time, $(\beta\gamma)_{jk}$ is the interaction of immigration and time, and ε_{ijk} is the random residual error. The sampling location was added to the model as a random factor. Furthermore, temporal trends were investigated using a segmented least squares linear regression to identify potential breakpoints in the slopes of species diversity and CPUE over time and test their temporal relationship with the accident in 2015. The statistical significance of the breakpoint was set at $p < 0.05$ and tested by respective confidence values of the slopes.

In order to examine changes in length class composition before and after the pollution event, catches per 1000 m river stretch were averaged (before: 2005–2013, after: autumn 2015 and spring 2016) for selected large fish species (barbel, chub, dace, common nase) in each severely affected sampling stretch. Fisher's exact test (Fisher, 1992) was used to test differences between the two periods at each sampling stretch.

To evaluate the success of directing the polluted water through the mill channels, differences in species diversity and CPUE were investigated, using GLMMs following the general formula:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij},$$

where Y_{ij} is the number of species or CPUE, μ is the overall mean, α_i denotes the type of water body, and ε_{ij} is the random residual error. The sampling location was added to the model as a random factor.

To evaluate the influence of fish-eating birds on CPUE before the incident, a GLMM with the following formula was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

where Y_{ijk} is logarithmized CPUE, μ is overall mean, α_i denotes the time point before (July 2005 to August 2008), with (September 2008 to March 2011) or after (April 2011 to June 2013) the lethal control measures, β_j denotes the threat status of examined fishes (according to the red list of fish species for the federal state Baden-Württemberg; Baer et al., 2014), $(\alpha\beta)_{ij}$ is the interaction of time point and threat status, and ε_{ijk} is the random residual error. The sampling location was added to the model as a random factor. For two endangered fish species (common nase, barbel), changes of the CPUE in each length class were compared between the different time points (before, with, and after culling measures) using Pearson's chi-squared test.

All analyses were performed with the help of the software JMP Pro Version 17.0.0 (64 bit, SAS Institute, Cary, USA).

3 Results

3.1 Fish Health

In autumn 2015, shortly after the accident, the gill tissues of more than 60% of examined fish exhibited moderate or severe pathological alterations (Fig. 3a, Fig. S3). Subsequent ordinal logistic regression revealed a statistically significant improvement in gill condition with time (whole model: 311 observations, $df = 1, 309$, $r^2 = 0.098$, $\mathrm{Chi}^2 = 77.824$, $p < 0.0001$), with predicted probabilities of milder alterations or normal gill condition increasing from 28% in autumn 2015 to 91% in autumn 2017 ($p < 0.0001$; Fig. 3a). The probability of severe gill impairment decreased from 38% initially to 2% at the end of the study period. The gill damage index also decreased significantly in accordance (273 observations, $df = 1, 271$, $r^2 = 0.289$, $F = 109.7179$, $p < 0.0001$) from 13.4 to 3.8 over the period of investigation (Fig. 3b).

A high prevalence of unidentified gill fluke (class Monogenea) and black spot disease (*Posthodiplostomum cuticola*) was observed directly after the accident and in spring 2016 (Fig. S3c–e). This prevalence decreased considerably thereafter with only single individuals showing gill flukes or black spot disease in autumn 2016 and from 2017 on. No difference in prevalence was observed between the river sections upstream and downstream of the discharge point.

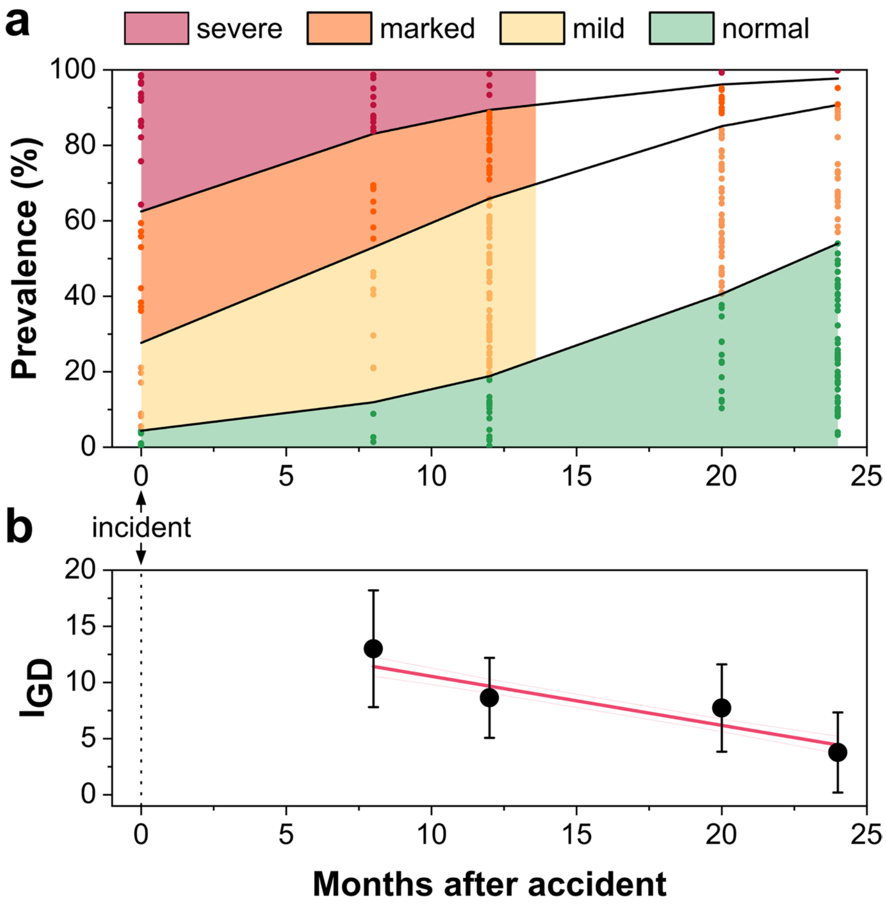

Fig. 3 Temporal trends in gill alterations observed in fish caught after the accident in the Jagst River between autumn 2015 and autumn 2017. **a** Fitted probability of gill condition based on ordinal logistic regression. **b** Mean (± standard deviation) gill damage index (I_{GD}) for each time point. Red line (± 95% confidence intervals) indicates the linear trend

3.2 Development of Fish Stock

The first survey, about 3 weeks after the fish kill in autumn 2015, revealed fish diversity ($p = 0.0004$) and the fish density ($p = 0.0007$) were significantly reduced for the first 25 km downstream of the point where artificial fertilizer had been discharged compared to the time period before the accident happened (Fig. S4, Table S4, and S5). In this severely affected stretch of the river, most sections yielded only one or two species with one outlier stretch yielding 8 (Table S1). Furthermore, for some species, i.e., chub, minnow, gudgeon, and stone loach, only single individuals were caught in autumn 2015, and all were small, less than 10 cm in body length. A similar result was seen in CPUE, where an average of only 26.3 individuals was detected per 100 m in the severely affected section after the accident, representing less than 5% of former fish density (Table S1, Fig. S4). The fishing results in spring 2016, 8 months after the accident and before the first stocking effort, showed no relevant recovery of the fish fauna in the severely damaged section, with species diversity and CPUE both remaining significantly lower than previously (species diversity: $p = 0.001$, CPUE: $p = 0.0005$; Table S4 and S5, Fig. S4). In autumn 2016 (12 months after the accident), some weeks after the first stocking effort, species diversity and CPUE had roughly doubled (Fig. S4). Later surveys revealed a steady increase in both parameters, and the segmented regression analysis identified significant breakpoints over time (Fig. 4b+d). Thereafter, the number of species and CPUE continued to increase steadily until autumn 2018, 36 months after the accident. Both values then plateaued—diversity at around pre-accident levels—but CPUE failed to reach previous levels (Fig. 4).

The utilized GLMMs revealed that both species diversity and CPUE increased significantly with the time since the accident (Table 2). Stocking had no

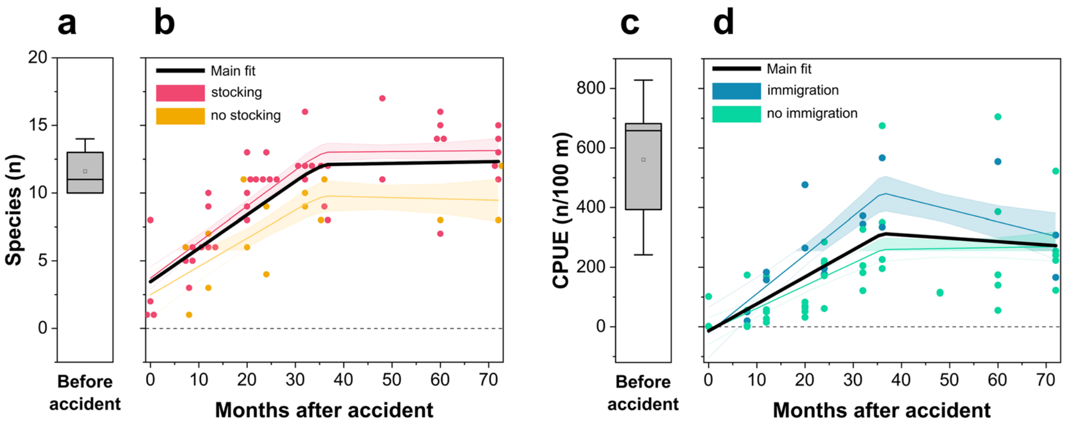

Fig. 4 Results of the segmented regression analysis investigating temporal changes in fish species diversity and CPUE in the severely damaged section of the Jagst River. **a** Boxplot species before the pollution event. **b** Calculated breakpoint regression for species number (black line) and descriptive illustration of differences for locations with and without stocking measures (colored lines). **c** Boxplot CPUE before the accident. **d** Calculated breakpoint regression for CPUE (black line) and descriptive illustration of differences for locations with and without natural immigration (colored lines). Points represent individual measurements, and colored bands indicate standard error

Table 2 Results of the general linear mixed models (GLMMs) utilized to analyze changes in species diversity and CPUE after the accident. Sampling location was added to the model as a random factor. *n*, number of observations; *df*, degrees of freedom. Statistically significant *p*-values are highlighted in bold

Analysis	Parameter	*n*	*df*	Model effect	F-value/r^2 adjusted[a]	*p*-value
Temporal trends	Species diversity	59	5, 53		*0.5167*[a]	**<0.0001**
				Stocking	11.3062	**0.0237**
				Immigration	2.8990	0.1561
				Time	19.3519	**<0.0001**
				Stocking x time	0.3924	0.5339
				Immigration x time	2.0932	0.1543
	CPUE	59	5, 53		*0.2151*[a]	**0.0045**
				Stocking	0.0533	0.8246
				Immigration	7.8432	**0.0293**
				Time	9.4420	**0.0034**
				Stocking x time	0.0109	0.9172
				Immigration x time	0.8224	0.8224
Main river vs. mill channels	Species diversity	20	1, 18		*0.3240*[a]	**0.0052**
	CPUE	20	1, 18		*0.3178*[a]	**0.0057**

effect on CPUE, but did relate to a statistically significant effect on the number of species recorded, with around 30% more species being present at stocked sites (least-square means ± standard error (SE): stocking = 10.77 ± 0.68, no stocking = 7.76 ± 0.97). Immigration had no effect on species number, but exerted a significant effect on CPUE, with around 40% higher CPUE at sites with natural immigration (least-square means ± SE: immigration = 313.91 ± 41.65, no immigration = 196.02 ± 27.36). No statistically significant effects were observed in interactions of stocking and time or immigration and time.

During monitoring in autumn 2016, after the first stocking event, nine out of 11 stocked species were recorded in the stocked sections, with only common nase and dace not detected. Subsequent surveys up until autumn 2018 indicated an increased density of all nine of these species, after which most plateaued. Of common nase and dace, only single individuals were found during the whole study period, even after two additional stockings in 2017 and 2018. Species of endemic fish which were not stocked, such as brown trout, pike (*Esox lucius*), perch, and carp, were recorded for the first time after the accident in the second year, spring 2017, but only as single individuals and densities of most of these species remained very low thereafter with only solitary individuals recorded where any were found at all. One non-endemic species, the stone moroko (*Pseudoraspora parva*), was found in several different river sections from autumn 2017 onwards. This and the occurrence of large individuals of certain species suggest that some additional stocking took place despite the moratorium agreed with local fishing clubs.

Small-sized fish species with low jumping and swimming ability, i.e., the bullhead (Fischer & Kummer, 2000), reappeared in sections 2 and 6 with immigration possibilities in autumn 2016, but were not recorded in section 5, the section without stocking and without obvious immigration routes until spring 2018, 2.5 years after the accident. Abundances of barbel and common nase, both large-sized species with high migration abilities, remained lower in the aftermath of the accident than before (mean ± SD of CPUE in 2018–2021: barbel = 10.4 ± 11.9, common nase = 1.2 ± 0.0; mean ± SD of CPUE in 2008–2014: barbel = 46.0 ± 67.6, common nase = 8.1 ± 18.7). Only barbel exhibited slowly increasing numbers in sections downstream of the accident, with notable deficits in length class contribution and natural recruitment.

Size class comparisons before and after the accident were not possible for most sampling sections because of extremely small catches in the severely damaged section of the Jagst River during the first two post-incident surveys (Table S1). Analysis for large growing fish species, such as barbel, chub, dace, and common nase, was possible only in the two most downstream sections, and here, statistically significant and striking differences (section 7: $p < 0.0001$, section 8: $p < 0.0001$) were apparent in size structure, with the larger size classes almost completely disappearing after the pollution event (Fig. 5).

Downstream of Elpershofen (Fig. 2, between sampling sections 6 and 7), great efforts were made to divert polluted water away from the main river and into adjacent mill channels. The utilized GLMMs revealed statistically significant preservation effects

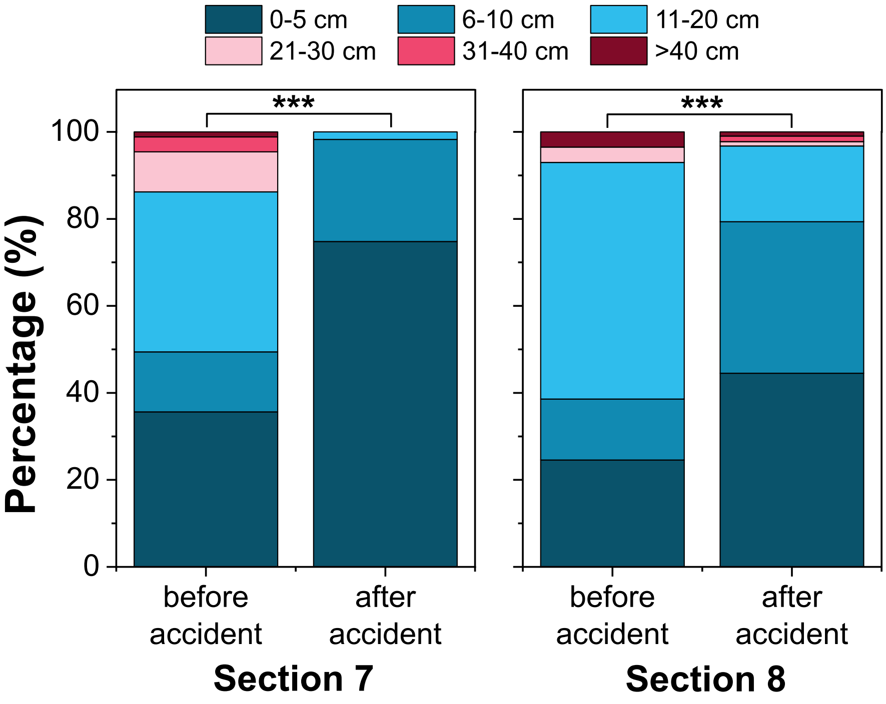

Fig. 5 Size class composition for larger growing species (>40 cm TL; here: barbel, chub, dace, and common nase) of the fish community in two stretches of the Jagst River before (2005–2013) and after (autumn 2015 and spring 2016) the pollution incident. Asterisks indicate statistically significant differences

of this measure when comparing the mill channel and the main river with respect to species diversity and CPUE (Table S6 and S7). In the main river, species number was around 35% higher ($F = 10.1075$, $p = 0.0052$) and CPUE about 72% higher ($F = 9.8504$, $p = 0.0057$), compared to the mill channels.

3.3 Cormorants

Before the incident, when comparing the CPUEs with or without (before and after) cormorant control measures, the utilized model (Table S7) revealed that control measures had a stark and statistically significant effect ($p < 0.0001$). CPUE was around 57% higher when cormorant control measures were in place than before (least-square means ± SE: before = 2.27 ± 1.42, during = 5.24 ± 1.41). After the measures stopped, CPUE dropped by around 70% (least-square means ± SE: after = 1.62 ± 1.50). The conservation status of examined fish species also had a statistically significant effect ($p < 0.0001$), in which CPUE of threatened species was around 77% lower than that of non-threatened species (least-square means ± SE: threatened = 1.29 ± 1.45, non-threatened = 5.55 ± 1.38). The interaction of control measures time point and threat status had no statistically significant effect ($p = 0.4709$). When comparing CPUE for different size classes before, during, and after cormorant control measures, statistically significant changes were observed for both common nase and barbel with middle size classes being distinctly underrepresented (Fig. 6).

After the pollution event, as a condition of derogations for lethal control of cormorants, monitoring was carried out on protected species of birds (Supplement Fig. S2). No interference or long-lasting effects (e.g., displacements) were recorded for target bird species. Cormorant counts on night roosts and results of the International Waterbird Census (IWC) reacted the same way and showed numbers of cormorants on the Jagst River in the winter 2020/21 remained high and unchanged. Between October 2018 and February 2019, the number of “cormorant days” was around 24,500 (on average, 163 cormorants every day). This likely resulted in an overall take of around 10 tons of fish (daily food intake 436 g according to Ridgway, 2010) roughly 42% of the estimated annual fishing yield of 23.8 tons (assumptions: 170 km Jagst River × 20 m river width ≈ 340 ha; fishery yield 70 kg ha^{-1}).

4 Discussion

4.1 Mortality After Incident

Ammonia is a highly toxic substance to fish, with direct impacts on fish health even at very low concentrations (Levit, 2010; U.S. EPA, 2013). The toxic effects vary according to several factors, mainly duration of exposure, dissolved ions, pH, water temperature, and fish size (Levit, 2010; U.S. EPA, 2013). All of these factors must be considered in understanding the massive fish kill that occurred in the Jagst River in the summer of 2015. It is highly likely that the dead fish found shortly after the accident succumbed

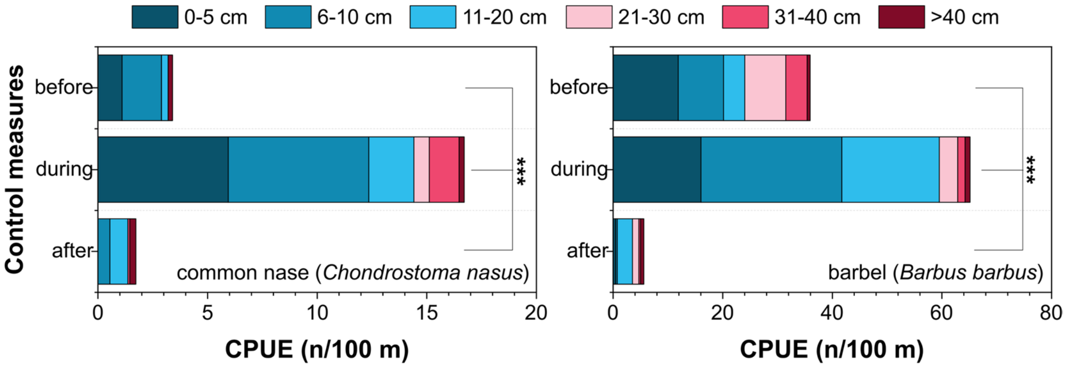

Fig. 6 Changes in CPUE by size class in common nase (left) and barbel (right) before, during, and after cormorant control measures. Asterisks indicate statistically significant differences with and without cormorant control measures (Pearson’s chi-squared test, *** = $p < 0.0001$)

to high levels of toxic unionized ammonia (NH_3), which leads to convulsions, coma, and death in all aquatic vertebrates (Randall & Tsui, 2002). The water temperature on the day of the catastrophe was 20–25 °C, pH values above 8 (data not shown), and high TAN loads all served to exacerbate the toxicity of the event (Table 3). It is surprising, given the severity of the spill, that the total mortality zone ended rather abruptly around 25 km downstream of the accident, despite the fact that even 40–50 km below the discharge point, NH_3 levels were still as high as 3–4 mg/L. This is several times above the levels listed by the U.S. EPA (2013) as acutely toxic for the fish present on the Jagst River (Table 3).

A number of factors may have contributed to this outcome. Local dilution by groundwater inlets and tributary brooks, which enter the Jagst River in the first 25 km, may have been significant. This is especially true since the accident happened at a time of low water when these small additions can contribute a large share of total flow during periods of low water, and offering small areas of refuge from the worst of the contamination. This consideration should place further emphasis on measures to protect groundwater levels (Perkin et al., 2017; Power et al., 1999). Secondly, damage may have been mitigated by a relatively short exposure time. The peak of the toxic ammonia wave had a residence time between several minutes and a few hours (velocity about 0.5 km h^{-1} according to LUBW, LAZBW, and Regierungspräsidium Stuttgart, 2017), while the acute toxicity tests in which LC50 values are based are carried out over a period of 96 h. Furthermore, given that fish pre-exposed to increased TAN levels in their natural habitat seem to be more susceptible to poisoning in contamination events (Soler et al., 2021), the normally negligible levels of TAN in the Jagst River may have helped maximize natural resilience. While most fish species cannot tolerate high environmental ammonia levels, acute susceptibility to ammonia in fish varies by species, size, and life stage. Some species are more tolerant to ammonia and have a variety of strategies to avoid the worst effects (Randall & Tsui, 2002) such as maintaining excretion (Randall et al., 1999) or conversion of ammonia to less toxic substances (Ip & Chew, 2010; Mommsen & Walsh, 1992; Peng et al., 1998). Both size and species effects were apparent in the aftermath of the Jagst River event, in that the small numbers of individual fish caught in autumn 2015 belonged to few species such as chub, minnow, gudgeon, and stone loach and were almost exclusively smaller than 10 cm. According to Levit (2010), susceptibility to ammonia decreases as fish develop from yolk sac fry to juvenile then increases with age thereafter.

No data about long-term mortality effects resulting from the incident on the Jagst River are available. However, in autumn 2015, around two-thirds of surviving fish exhibited gill damage, and these pathological alterations remained present at comparable levels after the first winter. Had significant numbers of these damaged fish died from this damage, the prevalence of injured fish would have declined, and therefore, significant undetected and lingering mortalities during the first year after the accident are deemed unlikely. An increase in the proportion of fish with healthy gills was observed in autumn 2016, but the data at hand is insufficient to determine whether this

Table 3 Acute toxicity of TAN at pH 7 (20 °C, 96 h) for different species according to U.S EPA (2013), calculated toxicity at pH 8 and respective calculated share of unionized ammonia

Species	Acute toxicity (TAN in mg/L) pH 7, 20 °C	Acute toxicity (TAN in mg/L) pH 8, 20°C	Unionized ammonia (NH_3 in mg/L)	Distance from discharge point (km)	Measured values of unionized ammonia (NH_3 in mg/L)
Threespine stickleback (*Gasterosteus aculeatus*)	282	28	1.18	0	6.40
Common carp (*Cyprinus carpio*)	106	10.6	0.44	25	3.20
Brown trout (*Salmo trutta*)	102	10	0.43	50	1.00
Rainbow trout (*Oncorhynchus mykiss*)	82	8	0.34	100	0.15

represents recovery or delayed mortality of damaged individuals (Cadiz & Jonz, 2020).

Our results suggest that action taken to divert polluted water of the accident through mill channels 15 km downstream was effective. While it is unfortunate that the fish in the mill channels suffered comprehensive mortality as a result of this action, these communities comprised mostly non-endangered species such as chub, roach, and bream. Meanwhile, the higher biodiversity of the natural fish communities in the parallel main river residual flows, including some endangered and protected species (like bullhead or barbel), was at least partially preserved. We assume that these protected sites subsequently served as sources of local repopulation and, along with stocking events, helped boost the natural recolonization of the damaged river sections.

4.2 Role of Stocking

Around 80 manmade barriers were constructed on the Jagst River over the years for water power and flood control (Ministry of the Environment, Climate Protection and the Energy Sector Baden-Württemberg, 2018) and have severely impaired the lateral and longitudinal continuity of the river, especially in the section most affected by the pollution event of 2015 (see Fig. S1b). These pre-existing obstructions hampered immigration, jeopardized the natural recovery of the fish population (Brinker et al., 2018), and increased the risk that an unoccupied habitat might be "overrun" by the first occurring species, especially if these happened to be r-strategists or worse invasives (Bajer & Sorensen, 2010), which might prevent recovery of a balanced and appropriate fish community. Given these concerns, a systematic translocation of local fish was deemed the only option for swiftly restoring lost fish species and re-establishing a natural fish community. The migratory and rheophilic common nase was almost completely wiped out during the pollution accident, with not even a handful of individuals captured in the most severely damaged section in the first surveys after the event. Common nase is considered a keystone species in the Jagst River and was thus a priority for re-establishment along with other migratory fish species like barbel (Lucas & Batley, 1996) and dace (Mann & Mills, 1986), and several small non-predatory fish species with low migration abilities such as bullhead (Fischer & Kummer, 2000) or stone loach (Tudorache et al., 2008). The steady increase and apparently balanced numbers of stocked fish species like bullhead and barbel recorded since then suggest the measure was effective for these species especially when these positive effects are compared with results in the unstocked section (Diembot, sampling section 5, Fig. 2, Table S1). Here, the recovery of the fish population was greatly delayed (bullhead was found for the first time 18 months after the accident), most likely due to the lack of lateral and longitudinal connectivity. In contrast, the unstocked section below the accidental discharge point (Lobenhausen, sampling section 2, Fig. 2, Table S1) recovered very quickly thanks to direct continuity with the undamaged section upstream. The same was observed in section 6, where the Jagst River is fed by a small tributary, the Brettach brook. The results strongly support the translocation of fish as a general recovery measure in sections of river where natural recolonization is physically hindered. However, in the case of common nase and dace, stocking in a variety of quantities and size classes yielded no improvement in the re-establishment. This disappointing result is in sharp contrast to other migratory species like barbel, for which stocking proved very effective. The reasons for the difference are not wholly clear, but common nase are known to be specialists of free-flowing river stretches, whereas barbel can also live in deeper and slowly flowing pools (Lucas & Batley, 1996; Ovidio et al., 2016; Ovidio & Philippart, 2008). Furthermore, common nase show a greater degree of homing behavior than barbel (Panchan et al., 2022). Thus, we hypothesize that common nase was already facing challenging habitat conditions due to the large number of existing barriers in the Jagst River. If this is the case, it may be that further measures are necessary to support functional fish migration, such as reducing water flow through bypasses to boost water flow in the main river (Petts, 1996). These additional measures should be urgently considered in the Jagst River in order to avoid the effective loss, in the near future, of this most iconic keystone fish species (Peňáz, 1996; Wetjen et al., 2020).

As some macroinvertebrates are very sensitive to high levels of ammonia (Berenzen et al., 2001), we also considered food availability as a potential limiting factor on stocking success. However, in the Jagst River, the effects of the incident on the macrozoobenthos were minor, and faunal similarity indices and species diversity showed no evidence of acute damage (LUBW, LAZBW, and Regierungspräsidium Stuttgart, 2017), rendering the possibility that stocked fish could not find enough food negligible.

4.3 Role of Cormorants

Cormorant predation has been shown already before the incident to exert substantial negative influence on certain endangered rheophilic species (Fig. 6, Table S7). The influence of predators on the recovery of a damaged fish stock in an exposed river like the Jagst River should be taken seriously. Cormorants were shown to exert significant feeding pressure on the river, drastically reducing fish density and affecting viable size classes. Over the past 10 years, the number of wintering cormorants on the Jagst River has increased steadily, and therefore, coordinated lethal control measures were carried out in the severely damaged section since 2016 in an attempt to protect fish stock recovery. These measures failed to bring about a reduction in cormorants (Fig. S2). On the contrary, the data at hand shows a clear increase in predation pressure on fish, whereas similar control measures enacted prior to the accident had previously been much more effective (Fig. 6, Table S7). In the first period, the number of cormorants was distinctly lower, both along the Jagst River and in the federal state of Baden-Württemberg than in the second period, so that the scared cormorants could more easily switch to other waters. Furthermore, setting a maximum number of cormorants to be culled independent of the effect of the measure, the percentage culled cormorants in relation to the total number decreased limited the necessary control options. It is estimated that in recent years, almost half of fish production on the Jagst River has been taken by cormorants, with a knock-on effect on the recovery potential of the fish community. Today, 7 years after the incident took place with water conditions comparable to those before the accident and most fish species returned to the river, recovery of the fish stock has stalled. Fish density has stagnated over 3 years at 20–50% of pre-accident levels, and individuals larger than 20 cm are severely underrepresented in the fish community (barbel, common nase, and dace densities 4.6, 1.1, and 1.1 individuals per 100 m for 2005–2013 compared to 0.6, 0.0, and 0.0 individuals per 100 m for 2019–2021, respectively). During winter, lower water temperatures slow the movements of fish enough to render them more easily caught by cormorants and birds clearly select larger prey at this time (Čech et al., 2008). The absence of any other increasing negative pressure on fish apparent in the Jagst River makes it highly likely that predation pressure from cormorants is the prime reason for continued low fish density on the river. A reduction in the cormorant numbers would most likely help to increase fish density and to establish more resilient stocks. As there were no indications of a long-term disturbance of priority bird species caused by cormorant control measures disturbance, the current measures might be stepped up.

4.4 Management Actions Initiated by the Catastrophe

As positive consequence of the catastrophe on the Jagst River has been a program of structural improvement measures begun in the years after the accident, including the modification of ground sills and the installation of fish passes (Ministry of the Environment, Climate Protection and the Energy Sector Baden-Württemberg, 2018). Other measures include the introduction of dead wood, boulders, and gravel to improve habitat structure and the restoration of side arms. A small number of weirs has been dismantled, but more than 40 are still in use to generate hydroelectric power and still present a barrier to the migration of fish, particularly in the most severely damaged section. The operators face a legal requirement to install fish passes under the water laws of Germany and the federal state of Baden-Württemberg. Unfortunately, residual water quantities are often not sufficient to allow such passes to operate effectively, and oversight by the appropriate authorities is often incomplete. In order to achieve the goals of the WFD (European Community, 2000), the federal state of Baden-Württemberg launched the program “Landesstudie Gewässerökologie” to plan structural improvement measures for water bodies. This planning has already been carried out for two sections of the Jagst River, including the section most severely damaged by the 2015 pollution event, and more will be implemented in the coming years. The actions taken so far have brought limited improvements to longitudinal connectivity and cross-linkage with tributaries, oxbows, and ditches and in securing groundwater levels and the benefits for fish, especially those endangered species most seriously affected, have been likewise small. In reality, the Jagst River in the affected area is more a concatenation of reservoirs and residual water bodies than a free-flowing river. It must be concluded that the measures implemented thus far are insufficient to achieve the stated aim of fundamentally improving resilience, especially with regard to the future threat and impacts of the climate change (Basen et al., 2022; IPCC, 2022). Posing severe for such a heavily disrupted habitat: immigration routes to cooler sections of the river and to smaller tributaries with more shaded areas were at best only partly accessible, and fish were forced to aggregate in deeper pools with greater exposure to fish-eating birds and other predators. While all the measures taken have certainly improved in situ conditions, actions to comprehensively and functionally restore the river are yet to be implemented or even fully conceived.

4.5 Lessons Learned

Accidents with the impact of the 2015 event on the Jagst River are isolated cases, but fish kills can be expected to increase in the future due to the exacerbating effects of climate change (Carere et al., 2023; IGB, 2022; La & Cooke, 2011). The current scarcity of data means that mistakes in assessing these situations and implementing *ad hoc* measures continue to be inevitable. It is our hope that the experiences on the Jagst River can help improve the response to comparable cases.

First of all, in the event of an identified fish kill, it is vital for the situation to be assessed to accurately determine the quantity and quality of the causing agent, i.e., by qualified analytical personnel with suitable equipment in close collaboration with aquatic specialists. In the present case, it was known that high oxygen levels mitigate the toxic effects of ammonia to aquatic organisms (Alabaster et al., 1979; Lloyd, 1961; Merkens & Downing, 1957), and consequently, a massive aeration program was implemented in the affected sections, day and night. Unfortunately, this was not a suitable measure, as (i) the positive oxygen effect is only effective in sublethal chronic cases (Downing & Merkens, 1955; U.S. EPA, 2013); (ii) the Jagst River is already supersaturated during the day with oxygen in summer, meaning the aeration unintendedly removed oxygen; and (iii) the disturbance caused additional stress to the already compromised fish. A second mitigation measure was to dilute Jagst River water from holding reservoirs without considering potential pH effects, which might have further boosted toxicity.

As a rule of thumb, it would be valuable to have an emergency plan established with contact points before an accident happens (Pintér, 1999). If, in the event of an accident, a cascade of responsible qualified and equipped persons is not at hand, precious time in which to take appropriate measures will be lost. Furthermore, the enlistment of external specialists from fields like toxicology, hydrology, and water chemistry is highly recommended, as sufficient expertise is unlikely to exist within regional administrations. These external experts can be seconded to help identify and quantify the causes of a disaster and swiftly forecast effects downstream, where relevant and responsible persons should be informed as soon as possible.

In cases where an accident such as that on the Jagst River does not cause long-term damage or deposits in the river bed, it may make sense to temporarily remove certain organisms, such as endangered mussels, crayfish, lampreys, or fish before the pollutant wave arrives. These organisms should be stored in suitable places for restocking once the water quality of their home river recovers. Fish could be placed in unaffected stretches or known other refuges, e.g., sections with groundwater inputs. Relocating aquatic species to nearby ponds, as happened at the Jagst River, is a short-term measure and only if water quality is adequate for the respective species. Due consideration should be given to the risk of diseases, parasites, and invasive alien species transmission if river fish are to be stored temporarily in fish ponds or other standing bodies of water.

The experiences on the Jagst River show that diverting water flow through mill channels can protect local biodiversity from the worst effects of pollution, especially as important specialist species tend to be associated with natural main channel, which act as refuges from which repopulation of autochthonous species can occur. However, the cutting off of residual sections in the main river in low water conditions without a suitable supplemental supply can itself pose a danger to fish, and any such measure should be closely monitored.

In a river that is segmented by dozens of small weirs and a lack of fish passes, natural fish immigration and recolonization are strongly impaired. While systematic stocking of the Jagst River with autochthonous species from sections above and below the damaged area was shown to significantly boost recovery of fish stocks, not all fish species showed positive population trends, and in particular, stocking the endangered common nase yielded no improvement in density or dispersal. For such sensitive and discerning species especially, advanced management measures, such as structural improvements, improvement of lateral and longitudinal connectivity as well as protection from predators might be necessary to restore endangered fish stocks.

Furthermore, after the accident in the Jagst River, local authorities, NGOs, fishing clubs, and members of the public wanted to help. This resource should be informed, coordinated, and organized by qualified persons in the fields of limnology, hydrology, nature chemistry, physiology, nature conservation, and fisheries management, and well-intentioned but counterproductive measures such as unofficial stocking with piscivorous or even non-endemic fish, such as the captured European catfish (*Silurus glanis*) and stone moroko after the accident, must be avoided.

To sum up, the most important protective measure is to bring the river ecosystem in question into a best possible resilient status, i.e., as close to natural as possible, and to prepare and resource a location-specific action plan involving qualified responsible and reachable people. Had this been the case for the Jagst River, action would have been taken faster and ineffective measures could have been avoided, resulting in more aquatic species being rescued.

Acknowledgements The authors offer sincere thanks to Oliver Koehler from the Langenburg fishing club and all other anglers who helped with the transfer and stocking measures as well as with the monitoring. We would like to thank numerous fishing clubs for allowing us to catch fish from their unaffected stretches of the Jagst River to stock them into the severely damaged section. The authors also thank Andreas Revermann and Hans-Peter Billmann for their assistance during the fieldwork. The language correction and improvement of the manuscript of Amy-Jane Beer are gratefully acknowledged. This work was supported by the Ministry of Agriculture Baden-Württemberg.

Author Contribution JGS, MS, JB, LI, and AB contributed to the study conception and design. Data collection and material preparation were performed by LI, JGS, MS, and JB, and analysis and data interpretation by JGS, MS, JB, SR, LI, and AB. The first draft of the manuscript was written by JGS, MS, JB, SR, and AB, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL.

Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

References

Alabaster, J. S., Shurben, D. G., & Knowles, G. (1979). The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon, *Salmo salar* L. *Journal of Fish Biology, 15*(6), 705–712. https://doi.org/10.1111/j.1095-8649.1979.tb03680.x

Antognazza, C. M., Andreou, D., Zaccara, S., & Britton, R. J. (2016). Loss of genetic integrity and biological invasions result from stocking and introductions of *Barbus barbus*: Insights from rivers in England. *Ecology and Evolution, 6*, 1280–1292. https://doi.org/10.1002/ece3.1906

Arthur, J. W., West, C. W., Allen, K. N., & Hedtke, S. F. (1987). Seasonal toxicity of ammonia to five fish and nine invertebrate species. *Bulletin of Environmental Contamination and Toxicology, 38*, 324–331. https://doi.org/10.1007/BF01606682

BAFU. (2020). *Berechnung von Schäden bei Fischsterben in Fliessgewässern*. Grundlage und Vorgehen. Bundesamt für Umwelt Umwelt-Vollzug Nr. 1912.

Baer, J., Blank, S., Chucholl, C., Dussling, U., & Brinker, A. (2014). Die Rote Liste für Baden-Württembergs Fische, Neunaugen und Flusskrebse. In *Report Ministry of Food, Rural Affairs and Consumer Protection Baden-Württemberg*. Retrieved January 9, 2022, from https://lazbw.landwirtschaft-bw.de/pb/,Lde/Startseite/Themen/Die+Rote+Liste+fuer+Baden-Wuerttembergs+Fische_+Neunaugen+und+Flusskrebse

Bajer, P. G., & Sorensen, P. W. (2010). Recruitment and abundance of an invasive fish, the common carp, is driven by its propensity to invade and reproduce in basins that experience wintertime hypoxia in interconnected lakes. *Biological Invasions, 12*, 1101–1112. https://doi.org/10.1007/s10530-009-9528-y

Basen, T., Ros, A., Chucholl, C., Oexle, S., & Brinker, A. (2022). Who will be where: Climate driven redistribution of fish habitat in southern Germany. *PLoS Clim, 1*(5), e0000006. https://doi.org/10.1371/journal.pclm.0000006

Berenzen, N., Schulz, R., & Liess, M. (2001). Effects of chronic ammonium and nitrite contamination on the macroinvertebrate community in running water microcosms. *Water Research, 35*(14), 3478–3482. https://doi.org/10.1016/s0043-1354(01)00055-0

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., & Wahli, T. (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases, 22*(1), 25–34. https://doi.org/10.1046/j.1365-2761.1999.00134.x

Brinker, A., Chucholl, C., Behrmann-Godel, J., Matzinger, M., Basen, T., & Baer, J. (2018). River damming drives population fragmentation and habitat loss of the threatened Danube streber (*Zingel streber*): Implications for conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems, 28*, 587–599. https://doi.org/10.1002/aqc.2878

Cadiz, L., & Jonz, M. G. (2020). A comparative perspective on lung and gill regeneration. *Journal of Experimental Biology, 223*(19), jeb226076. https://doi.org/10.1242/jeb.226076

Carere, M., Lacchetti, I., di Domenico, K., Cristiano, W., Mancini, L., De Felip, E., Ingelido, A. M., Massei, R., & Tancioni, L. (2023). Fish-kills in the urban stretch of the Tiber River after a flash-storm: Investigative monitoring with effect-based methods, targeted chemical analyses, and fish assemblage examinations. *Water, Air, & Soil Pollution, 234*, 159. https://doi.org/10.1007/s11270-023-06102-4

Čech, M., Čech, P., Kubečka, J., Prchalová, M., & Draštík, V. (2008). Size selectivity in summer and winter diets of Great Cormorant (*Phalacrocorax carbo*): Does it reflect season-dependent difference in foraging efficiency? *Waterbirds, 31*(3), 438–447. https://doi.org/10.1675/1524-4695-31.3.438

Chucholl, C., Baer, J., Brinker, A., Hartmann, F., Bartl, G., Glönkler, F., Künemund, F., Weisser, P., Konrad, M., Dussling, U., & Geray, D. (2019). *Fischökologisch bedeutsame Gewässer in Baden-Württemberg*. Ministerium für Ländlichen Raum und Verbraucherschutz Baden-Württemberg. Retrieved January 9, 2022, from https://fortbildung-lazbw.lgl-bw.de/lazbw/webbasys/index.php?kathaupt=601&dsnr=58&kathauptalt=600

Downing, K. M., & Merkens, J. C. (1955). The influence of dissolved-oxygen concentration on the toxicity of un-ionized ammonia to rainbow trout (*Salmo gairnderii* Richardson). *Annals of Applied Biology, 43*, 243–246. https://doi.org/10.1111/j.1744-7348.1955.tb02472.x

Dunn, O. J. (1964). Multiple comparisons using rank sums. *Technometrics, 6*(3), 241–252. https://doi.org/10.1080/00401706.1964.10490181

Dunnett, C. W. (1955). A multiple comparisons procedure for comparing several treatments with a control. *Journal of the American Statistical Association, 50*, 1096–1121. https://doi.org/10.2307/2281208

Dussling, U. (2009). *Handbuch zu fiBS. Schriftenreihe des Verbandes Deutscher Fischereiverwaltungsbeamter und Fischereiwissenschaftler e.V., no. 15*. Retrieved January 9, 2022, from https://lazbw.landwirtschaft-bw.de/pb/site/pbs-bw-new/get/documents/MLR.LEL/PB5Documents/lazbw_2017/lazbw_ffs/Dokumente_ffs/WRRL/Handbuch_fiBS.pdf

Emerson, K., Russo, R. C., Lund, R. E., & Thurston, R. V. (1995). Aqueous ammonia equilibrium calculations: Effect of pH and temperature. *Journal of the Fisheries Research Board of Canada, 32*(12), 2379–2383. https://doi.org/10.1139/f75-274

Community, E. (2000). Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official Journal of the European Communities, L327*, 1–72.

Fischer, S., & Kummer, H. (2000). Effects of residual flow and habitat fragmentation on distribution and movement of bullhead (*Cottus gobio* L.) in an alpine stream. *Hydrobiologia, 422-423*, 305–317. https://doi.org/10.1023/A:1017083714513

Fisher, R. A. (1992). In S. Kotz & N. L. Johnson (Eds.), *Breakthroughs in statistics*. Springer Series in Statistics. *Statistical methods for research workers*. Springer. https://doi.org/10.1007/978-1-4612-4380-9_6

Francis-Floyd, R., Watson, C., Petty, D., & Pouder, D.B. (2009). Ammonia in aquatic systems. *UF/IFAS University of Florida (UF)/Institute of Food and Agricultural Sciences (IFAS)*, FA, *16*, 1-4. https://doi.org/10.32473/edis-fa031-2022

IGB. (2022). *Suspicion confirmed: Algal toxin produced by brackish water species detected in Oder water – natural causes unlikely*. Press release, Leibniz Institute of Freshwater Ecology and Inland Fisheries. Retrieved January 17, 2022, from https://www.igb-berlin.de/en/news/suspicion-confirmed-algal-toxin-produced-brackish-water-species-detected-oder-water

IPCC. (2022). Climate Change 2022: Impacts, adaptation and vulnerability. In H.-O. Pörtner, D. C. Roberts, M. Tignor, E. S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, & B. Rama (Eds.), *Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (p. 3056). Cambridge University Press. https://doi.org/10.1017/9781009325844

Ip, Y. K., & Chew, S. F. (2010). Ammonia production, excretion, toxicity, and defense in fish: A review. *Frontiers in Physiology, 1*, 134. https://doi.org/10.3389/fphys.2010.00134

La, V. T., & Cooke, S. J. (2011). Advancing the science and practice of fish kill investigations. *Reviews in Fisheries Science, 19*(1), 21–33. https://doi.org/10.1080/10641262.2010.531793

Levene, H. (1960). Robust tests for equality of variances. In *Contributions to probability and statistics* (pp. 278–292). Stanford University Press.

Levit, S. M. (2010). A literature review of effects of ammonia on fish. *Montana*. https://www.conservationgateway.org/ConservationByGeography/NorthAmerica/UnitedStates/alaska/sw/cpa/Documents/L2010ALR122010.pdf

Lloyd, R. (1961). Effect of dissolved oxygen concentrations on the toxicity of several poisons to rainbow trout (*Salmo gairdnerii* Richardson). *Journal of Experimental Biology, 38*(2), 447–455. https://doi.org/10.1242/jeb.38.2.447

LUBW, LAZBW & Regierungspräsidium Stuttgart (2016). *Fischsterben in der Jagst - Vorläufige Abschätzung der ökologischen Auswirkungen des Großbrandes in der Lobenhausener Mühle*. Report. Retrieved February 8, 2022, from https://pudi.lubw.de/detailseite/-/publication/77553

LUBW, LAZBW & Regierungspräsidium Stuttgart (2017). *Ökologische Auswirkungen des Großbrandes in der Lobenhausener Mühle auf die Jagst. Final report*. Retrieved February 8, 2022, from https://pudi.lubw.de/detailseite/-/publication/24380

Lucas, M. C., & Batley, E. (1996). Seasonal movements and behaviour of adult barbel *Barbus barbus*, a riverine cyprinid fish: Implications for river management. *Journal of Applied Ecology, 33*, 1345–1358. https://doi.org/10.2307/2404775

Mann, R. & Mills, C. (1986). *Biological and climatic influences on the dace Leuciscus leuciscus in a southern chalk-stream*. Annual Report, Freshwater Biological Association, Ambleside. Retrieved February 23, 2022, from https://aquadocs.org/handle/1834/22759

Merkens, J. C., & Downing, K. M. (1957). The effect of tension of dissolved oxygen on the toxicity of un-ionized ammonia to several species of fish. *Annals of Applied Biology, 45*, 521–527. https://doi.org/10.1111/j.1744-7348.1957.tb05891.x

Ministry of the Environment, Climate Protection and the Energy Sector Baden-Württemberg (2018). *Aktionsprogramm Jagst - Bilanz und Ausblick*. Fact Sheet. Retrieved Februaray 8, 2022, from http://www.um.baden-wuerttemberg.de

Mommsen, T. P., & Walsh, P. J. (1992). Biochemical and environmental perspectives on nitrogen metabolism in fishes. *Experientia, 48*, 583–593. https://doi.org/10.1007/BF01920243

Ovidio, M., Hanzen, C., Gennotte, V., Michaux, J., Benitez, J.-P., & Dierckx, A. (2016). Is adult translocation a credible way to accelerate the recolonization process of *Chondrostoma nasus* in a rehabilitated river? *Cybium, 40*, 43–49. https://doi.org/10.26028/cybium/2016-401-004

Ovidio, M., & Philippart, J. C. (2008). Movement patterns and spawning activity of individual nase *Chondrostoma nasus* (L.) in flow-regulated and weir-fragmented rivers. *Journal of Applied Ichthyology, 24*, 256–262. https://doi.org/10.1111/j.1439-0426.2008.01050.x

Panchan, R., Pinter, K., Schmutz, S., & Unfer, G. (2022). Seasonal migration and habitat use of adult barbel (*Barbus barbus*) and nase (*Chondrostoma nasus*) along a river stretch of the Austrian Danube River. *Environmental Biology of Fishes, 105*, 1601–1616. https://doi.org/10.1007/s10641-022-01352-3

Peňáz, M. (1996). *Chondrostoma nasus* - its reproduction strategy and possible reasons for. In A. Kirchhofer, D. R. Müller, & D. Hefti (Eds.), *Conservation of endangered freshwater fish in Europe* (pp. 279–297). Springer Science & Business Media.

Peng, K. W., Chew, S. F., Lim, C. B., Kuah, S. S. L., Kok, W. W., & Ip, Y. K. (1998). The mudskippers *Periophthalmodon schlosseri* and *Boleophthalmus boddaerti* can tolerate environmental NH_3 concentrations of 446 and 36 µM, respectively. *Fish Physiology and Biochemistry, 19*(1), 59–69. http://scholarbank.nus.edu.sg/handle/10635/101971

Perkin, J. S., Gido, K. B., Falke, J. A., Fausch, K. D., Crockett, H., Johnson, E. R., & Sanderson, J. (2017). Groundwater declines are linked to changes in great plains stream fish assemblages. *Proceedings of the National Academy of Sciences of the United States of America, 114*, 7373–7378. https://doi.org/10.1073/pnas.1618936114

Petts, G. E. (1996). Water allocation to protect river ecosystems. *Regulated Rivers: Research & Management, 12*, 353–365. https://doi.org/10.1002/(SICI)1099-1646(199607)12:4/5<353::AID-RRR425>3.0.CO;2-6

Pintér, G. G. (1999). The Danube accident emergency warning system. *Water Science and Technology, 40*(10), 27–33. https://doi.org/10.1016/S0273-1223(99)00669-1

Poleksić, V., & Mitrović-Tutundžić, V. (1994). Fish gills as a monitor of sublethal and chronic effects of pollution. In R. Muller & R. Lloyd (Eds.), *Sublethal and chronic effects of pollutants on freshwater fish* (pp. 339–352). Fishing News Books for FAO.

Power, G., Brown, R. S., & Imhof, J. G. (1999). Groundwater and fish—Insights from northern North America. *Hydrological Processes, 13*, 401–422. https://doi.org/10.1002/(SICI)1099-1085(19990228)13:3<401::AID-HYP746>3.0.CO;2-A

Randall, D., Wilson, J., Peng, K., Kok, T., Kuah, S., Chew, S., Lam, T. J., & Ip, Y. K. (1999). The mudskipper, *Periophthalmodon schlosseri*, actively transports NH_4^+ against a concentration gradient. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 277*, R1562–R1567. https://doi.org/10.1152/ajpregu.1999.277.6.R1562

Randall, D. J., & Tsui, T. K. N. (2002). Ammonia toxicity in fish. *Marine Pollution Bulletin, 45*, 17–23. https://doi.org/10.1016/S0025-326X(02)00227-8

Regierungspräsidium Stuttgart (2021). *EU – Wasserrahmenrichtlinie Bericht zur Bestandsaufnahme Bearbeitungsgebiet Neckar.* Teilbearbeitungsgebiet 48 (Jagst), Textband. Retrieved Februaray 8, 2022, from https://rp.baden-wuerttemberg.de/fileadmin/RP-Internet/Themenportal/Wasser_und_Boden/Wasserrahmenrichtlinie/TBG_48_-_Jagst/_DocumentLibraries/WRRL_48_Begleit_2022-27/TBG48_Begleitdokumentation.pdf

Ridgway, M. S. (2010). A review of estimates of daily energy expenditure and food intake in cormorants (*Phalacrocorax* spp.). *Journal of Great Lakes Research, 36*, 93–99. https://doi.org/10.1016/j.jglr.2009.10.002

Sachs, L. (2004). *Angewandte Statistik*. Springer-Verlag. https://doi.org/10.1007/978-3-662-05744-5

Soler, P., Faria, M., Barata, C., García-Galea, E., Lorente, B., & Vinyoles, D. (2021). Improving water quality does not guarantee fish health: Effects of ammonia pollution on the behaviour of wild-caught pre-exposed fish. *PLoS One, 16*, e0243404. https://doi.org/10.1371/journal.pone.0243404

Tudorache, C., Viaene, P., Blust, R., Vereecken, H., & De Boeck, G. (2008). A comparison of swimming capacity and energy use in seven European freshwater fish species. *Ecology of Freshwater Fish, 17*, 284–291. https://doi.org/10.1111/j.1600-0633.2007.00280.x

U.S. EPA (2013). *Aquatic life ambient water quality criteria for ammonia- freshwater. United States Environmental Protection Agency, Washington DC, EPA-822-R-13-001*. EPA, Washington. Retrieved January 9, 2022, from https://www.epa.gov/sites/default/files/2015-08/documents/aquatic-life-ambient-water-quality-criteria-for-ammonia-freshwater-2013.pdf

Wetjen, M., Hübner, D., Seehausen, O., & Schulz, R. (2020). Genetic diversity of endangered *Chondrostoma nasus* in the River Rhine system: Conservation genetics considerations on stocking and reintroduction. *Knowledge and Management of Aquatic Ecosystems, 421*, 25. https://doi.org/10.1051/kmae/2020016