Acta Ichthyologica et Piscatoria 54, 2024, 165–176 | DOI 10.3897/aiep.54.124289

PENSOFT.

ACTA ICHTHYOLOGICA ET PISCATORIA

Can stocking with advanced European grayling fry strengthen its populations in the wild?

Mladen AVRAMOVIĆ[1], Jan TUREK[1], Pavel LEPIČ[1], Mariusz SZMYT[2], Jan PASTEJŘÍK[1], Tomáš RANDÁK[1]

1 *University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vodňany, Czech Republic*

2 *Department of Ichthyology and Aquaculture, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland*

https://zoobank.org/0F5CB4E7-2D3C-43A0-B333-9F60054317F5

Corresponding author: Mladen Avramović (mavramovic@frov.jcu.cz)

Academic editor: Predrag Simonović ♦ **Received** 30 March 2024 ♦ **Accepted** 19 June 2024 ♦ **Published** 2 August 2024

Citation: Avramović M, Turek J, Lepič P, Szmyt M, Pastejřík J, Randák T (2024) Can stocking with advanced European grayling fry strengthen its populations in the wild? Acta Ichthyologica et Piscatoria 54: 165–176. https://doi.org/10.3897/aiep.54.124289

Abstract

European grayling, *Thymallus thymallus* (Linnaeus, 1758) (Actinopterygii: Salmoniformes: Salmonidae), represents one of the highly attractive riverine fishing species in Europe. Its populations have declined in the Czech Republic due to various adverse factors. Current approaches for strengthening these populations based on restocking with artificially reared 1+ or 2+ old individuals have proven to be ineffective. This study focuses on the possibilities of supporting or restoring these populations by reintroducing two-month-old, fast-growing fry reared in ponds. In June 2021, 5400 advanced fry marked with Alizarin Red (ARS) were introduced into three free-flowing South Bohemian streams. The recapture rates and biometric data (length and weight) of stocked grayling were assessed at the release sites as well as further downstream. The first electrofishing monitoring was conducted at the end of the growing season (October) and after their first winter (March). Water temperature and flow rate at the stocking sites were monitored throughout the growing season. The substantial number of recaptured individuals across all monitored sites suggests that the fry successfully adapted to their new habitat and significantly contributed to the composition of local fish communities. Downstream movement of stocked fry correlated with lower water temperatures and higher flows. Our findings demonstrate that some introduced individuals successfully overwintered at all three reintroduction sites. Marking with ARS has proven to be a very effective non-invasive method of group marking juvenile fish and is suitable for monitoring stocking programs. The production and stocking of fast-growing advanced fry of European grayling is thus a promising strategy for revitalizing and strengthening the populations of this threatened fish in running waters.

Keywords

adaptability of stocked fish, ARS marking, grayling stocks production, natural discharge, restocking grayling

Introduction

The European grayling, *Thymallus thymallus* (Linnaeus, 1758) (Actinopterygii: Salmoniformes: Salmonidae) (thereafter referred in the text as EG), represents an attractive recreational fishing species that fell under fisheries management programs in the mid-20th century (Janković 1964; Northcote 1995). Nowadays, the wild population has declined dramatically through its natural range due to flow regulation and water quality degradation (Northcote 1995; Thorfve and Carlstein 1998; Thorfve 2002; Turek et al. 2012), hydropeaking (Hayes et al. 2021), and severe bird predation (Jepsen et al. 2018). Despite efforts to counter this decline, restocking projects, usually based on restocking with farmed 1+ and 2+-year-old fish, have proven ineffective, resulting in notably low recapture rates in natural habitats (Turek et al. 2012, 2018; Avramović et al. 2024). Consequently, exploring different release sizes of stocked fish is recommended for more effective restocking (Lorenzen 2005). Specifically, the release of early stages (0+) could improve stocking success (Carlstein 1997; Czerniawski et al. 2015) and mitigate the development of rearing-related traits in salmonids, which often hinders their survival in natural environments (Fraser et al. 2011).

Rearing techniques influence post-stocking recaptures of EG (Carlstein 1997). During the early stages, grayling can be fed with zooplankton (Luczynski et al. 1986) to aid their successful adaptation in the wild (Czerniawski et al. 2015). Supplementing or fully transitioning to dry pellets in the first summer can enhance survivability in ponds (Carlstein 1993) and lead to satisfactory post-stocking biometrics for grayling in lotic water bodies (Carlstein 1997). However, the stocking programs with pond-reared EG fry fed on the zooplankton remain unassessed in natural riverine habitats.

Along with the tendency of stocked grayling to drift downstream (Thorfve and Carlstein 1998; Horká et al. 2015), the extent of fish disappearance from stocking sites is supposed to correspond to the habitat capacity. Thus, mass stocking events can lead to density-dependent mortality due to increased competition (Hühn et al. 2014; Lorenzen and Camp 2019). Furthermore, the viability of stocking 0+ stages is uncertain due to potential mortality caused by environmental factors (Lorenzen and Camp 2019), which play a crucial role in determining outcomes of stocking salmonids (Brignone et al. 2022). Rapid increases in water flow in small streams increase the risk of young salmonids being swept away (Jowett and Richardson 1989; Hayes et al. 2010; Warren et al. 2015). In cold water, these challenges further hinder fish as they impair their swimming abilities (Enders et al. 2008) and increase the risk of stranding (Poff et al. 1997; Auer et al. 2017, 2023).

In general, the growth of EG 0+ has to be optimized during the main growing season by low discharge and warm temperatures (Marsh et al. 2022). The optimal growth temperature for EG has been identified as 17.3°C (Mallet et al. 1999) and higher temperatures are supposed to reduce growth (Persat and Pattee 1981) and recruitment of 0+ stages (Charles et al. 2006). Additionally, stocking in summer may encounter high diel amplitudes in water temperature (T_a) that significantly affect fish performance (Malcolm et al. 2008). These amplitudes tend to affect juveniles more than adults (Auer et al. 2023) and lead to increased metabolic costs (Beauregard et al. 2013) that have a negative impact on salmonid growth (Meeuwig et al. 2004). In winter, predation by cormorants can severely affect grayling populations (Jepsen et al. 2018), while in shallower streams herons also pose a threat and cause significant losses amongst stocked fish (Miyamoto et al. 2017). Submerged wooden structures can help reduce predation rates (Miyamoto et al. 2017) and boost fish site fidelity during this period (Watz 2017).

Any assessment of the success of a stocking program must include the monitoring of post-stocking recapture rates and fish performance (Waples et al. 2007), with the precondition that appropriate fish marking for subsequent stocked fish detection such as Alizarin Red (ARS) has been employed (Hühn et al. 2014; Halačka et al. 2018). In the South Bohemian streams in the Czech Republic, the abundance of EG was found to be concerningly low, indicating the urgent demand for restocking. Therefore, the aims of this study were:

- To establish a rearing and monitoring program of advanced grayling fry to aid natural population recovery.
- To evaluate if stocked fish would adapt successfully and result in acceptable recapture rate, and lengths and weights that would mirror those of natural streams.
- To assess displacement of grayling fry from the release site.
- To describe stocking outcomes in relation to the water temperature and discharge throughout the growing season.
- To provide valuable recommendations for future EG reintroductions.

We assumed that the stocked fish would adapt successfully. Due to their previous experiences of living in a pond with natural prey and the fact that the stocking took place in the middle of the growing season, we predicted acceptable recapture rates and body growth rates in natural streams. Also, our study discusses stocking outcomes in relation to the water temperature and discharge throughout the growing season.

Material and methods

Rearing of European grayling advanced fry. A total of 20 000 grayling fry were purchased from the hatchery Kachní farma Holýšov and transported on 18 May 2021 to a small pond (0.1 ha) with maximum depths of 100–120 cm situated in the experimental pond area of the Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice (FFPW USB) in Vodňany. The water chemistry parameters (pH, O_2 [mg · L^{-1}]) were monitored using the WTW pH 3310 and WTW oxi 3205 equipment, respectively (Table 1). The water temperature was monitored in the rearing pond with dataloggers (Minikin Tie, Ø = 20 mm, www.emsbrno.cz) (Table 1). The grayling fry initially consumed just natural food in the pond until zooplankton were continuously added. The pond's water temperature exhibited an exponential increase from 18 May to 28 June 2021 during the rearing period, with a mean temperature of 18.7°C (SD ± 3.3). The pond rearing program finished at the time when the final ten days of pond water temperatures had a mean of 22.8°C.

Table 1. Basic water parameters measured on the day of harvesting in the pond and in the three stocking stretches (28 June) the day before release. The fish (European grayling, *Thymallus thymallus*) gradually acclimatized over 24 h to the water temperature of ~17°C.

Site	Dissolved O_2 [$mg \cdot L^{-1}$]	pH	Temperature [°C]
Pond	9.51	8.03	24.3
Chvalšinský	8.60	8.05	17.7
Blanice	9.40	7.72	17.2
Zlatý	9.02	7.65	16.2

After approximately six weeks of rearing, 5400 grayling fry had survived in the pond out of the initial 20 000 stocked fry, with a survival rate of 27%. The mean standard length of the fry was 5.43 ± 0.34 cm (n = 100, mean ± SD), with a mean weight of 2.2 ± 0.39 g, and the mean Fulton's condition factor ($K = 100 \times (W \cdot L^{-3})$) of 0.82 ± 0.07. We translocated the fry into a tank where mass-marking was performed using ARS. The chosen methodology for marking the 0+ fish (Halačka et al. 2018) was a one-hour free-swimming session in an aerated dilution of ARS 150 $mg \cdot L^{-1}$. The next day (29 June), fish were divided into three equal groups (3 × 1800 fry) and driven (~30 min) in aerated tanks filled with water at temperatures that matched the selected natural streams. No fish mortality was observed after either the mass-marking or transportation. The fish were released at the upstream-most location of the selected stretches of river.

Stocking sites. All three chosen stocking sites are natural, free-flowing water bodies with no artificial flow regulation upstream from the release sites (Fig. 1). Their discharge regimes are largely determined by rainfall dynamics and subsequent runoffs. Besides the typical cold-water species, a considerable number of thermophilic species in the Chvalšinský and Zlatý streams originated from the upstream inflow of nearby ponds or, in the case of Blanice, had migrated upstream from the Husinec Reservoir.

The river Blanice is the most important right-hand tributary of the Otava, which it joins at the village of Putimi at river km 32.28. The total length of the Blanice River is 94.73 km and it has a catchment area of 861.91 km^2. The stretch in which the graylings were stocked is located approximately 1 km upstream from the reservoir inflow point at river km 60 (37 ha; 2.5×10^6 m^3). The stocking stretch has a steeper gradient and higher velocity than the other localities used in this study. It is 170 m long, with a mean width of 10.5 m (9–12 m) and depths in the range of 20–80 cm. The study site lies at an altitude of 570 m above sea level (GPS: 49.0334836°N, 13.9645994°E) and has a mean annual flow of 1.8 $m^3 \cdot s^{-1}$. The Blanice River runs through a mountain range surrounded by tall coniferous forests, while the deciduous trees and bushes form a riparian buffer along the stream. The riverbed is rich with large rocks that help form numerous pockets or pools—hence the local name of this stretch of the Blanice as "pocket water"—and the remaining bottom materials are pebbles and gravel, which also form the banks.

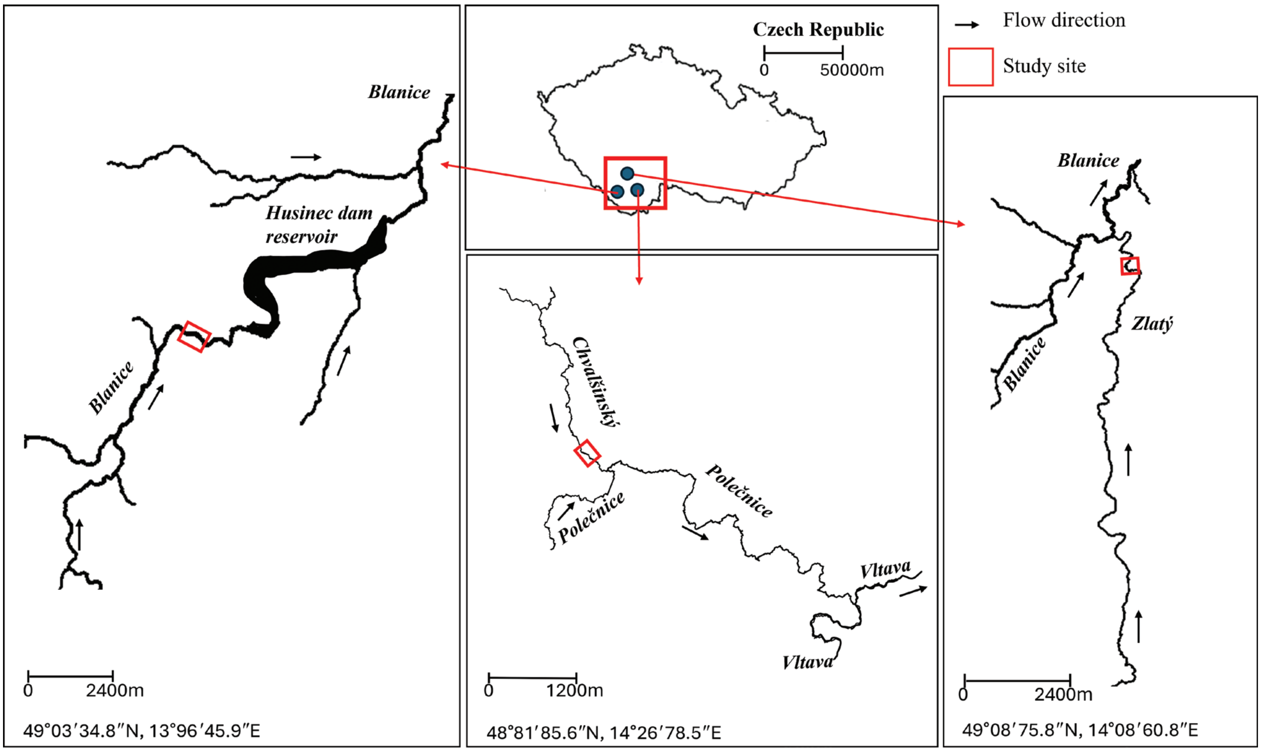

Figure 1. Release sites of European grayling, *Thymallus thymallus*, in three streams: Blanice, Chvalšinský, and Zlatý in the Czech Republic.

Chvalšinský potok (Polečnice) is a river in the Český Krumlov district, a left-bank tributary of the Vltava River. Its total length is 32.8 km and has a catchment area of 197.9 km^2. Grayling were released into a section of the river located below Kájov village, approximately 5.5 km upstream from where it joins the Vltava (GPS: 48.8185678°N, 14.2678514°E). This stocking stretch is a typical riffle-and-pool type stream, with a mean annual flow of 1.1 m^3 · s^{-1}. This sequence is repeated along the stream and there are fast rapids along narrow stretches with larger stones, followed by sections of long, deep, and calm pools with a sand and gravel streambed. The stretch length is 180 m, with a mean width of 3.5 m (2–5 m) and depths in the range of 10–100 cm. The site is at an altitude of 519 m above sea level. Generally, the banks are moderately steep, although some stretches have one steep eroded bank with a flat opposite bank formed from gravel and sand deposition (sand bar). Upstream from the study site, there is a small village whose wastewater probably increases the risk of eutrophication. The study site is surrounded by deciduous forest and cultivated meadows, and along the stream, the riparian vegetation has formed well-developed canopy cover.

The Zlatý stream is the longest tributary of the river Blanice in South Bohemia, into which it flows at river km 41. The total length of this stream is 36.7 km and it has a catchment area of 92.3 km^2. The section where the grayling were stocked is located near the village of Šipoun, 3 km upstream from the confluence with the river Blanice (GPS: 49.0875896°N, 14.0860800°E). Relatively deep pools, weak rapids, and shallow gradients characterize the chosen stretch of this stream. The length of the chosen stretch is 200 m, with a mean width of 2.25 m (1.5–3 m) and depths in the range of 10–160 cm. The streambed is sandy to gravelly, with a layer of clay deposits in places, and a slow current. The mean annual flow here is 0.51 m^3 · s^{-1}. It flows through a plain with meadows forming meanders and has a well-developed riparian vegetation dominated by willows of various ages. The low dense canopy is tunnel-like in some parts since the root systems extend into the stream and provide shelter for fish. The site is at an altitude of 445 m above sea level. It is distinguished from the previous two streams by its steeper banks, as well as by its considerable clay deposits and higher turbidity. The high discharge does not seem to have any significant impact on the width of this stream.

The fish communities vary between stretches (Fig. 2) but have in common brown trout, *Salmo trutta* Linnaeus, 1758, as the dominant salmonid species. Beside EG and brown trout, other fish species present at the release sites were: brook trout, *Salvelinus fontinalis* (Mitchill, 1814); perch, *Perca fluviatilis* Linnaeus, 1758; chub, *Squalius cephalus* (Linnaeus, 1758); dace, *Leuciscus leuciscus* (Linnaeus, 1758); roach, *Rutilus rutilus* (Linnaeus, 1758); bullhead, *Cottus gobio* Linnaeus, 1758; common carp, *Cyprinus carpio* Linnaeus, 1758; stone loach, *Barbatula barbatula* (Linnaeus, 1758); stone moroko, *Pseudorasbora parva* (Temminck et Schlegel, 1846); gudgeon, *Gobio gobio* (Linnaeus, 1758); burbot, *Lota lota* (Linnaeus, 1758); Eurasian minnow, *Phoxinus phoxinus* (Linnaeus, 1758); pikeperch, *Sander lucioperca* (Linnaeus, 1758); and river lamprey, *Lampetra fluviatilis* (Linnaeus, 1758). Lastly, it is important to note that otters and herons prey heavily on fish stocks in these stretches, and predation pressure from larger fish, especially brown trout, can be assumed to occur also.

Sampling. On 28 June 2021, the fish populations along chosen stretches (150 m) of selected natural streams were monitored via an electrofishing survey to provide data about the composition of fish communities. We performed single-pass electrofishing by using two back-pack generators with pulsed-DC (FEG 1500, EFKO-Germany) and other standard equipment for electrofishing. Also, two additional back-pack pulsed-DC electrofishing units (FEG 3000, EFKO-Germany) were placed at the upper border of each site to prevent fish from escaping upstream. The depth of the studied stretches varied, and we used corresponding cathode lengths of 100–150 cm, while the anode hope diameter was 30 cm. The streams' conductivities were within the range of 140–260 µS · cm^{-1}. The setting parameters of generators entirely corresponded to the character and conductivity of the monitored streams.

The post-stocking electrofishing survey was performed in the stocking stretches approximately 3.5 months (105 days) after stocking (11 October 2021). We used the same electrofishing method and equipment as we used for pre-stocking monitoring. The composition of the fish communities was monitored in the same stretches as before stocking took place. Additionally, we monitored 300 m of the river downstream from the stocking site to detect the displacement of stocked fish. The detection of ARS fluorescent marks on the fins of all caught grayling juveniles was checked by using laser pointers (green, ʎ = 532 nm, 50 mW, www.eclipsera.cz) and laser protection glasses (ʎ = 190–540 nm, www.eclipsera.cz). For continuous measuring of the stream temperature at 30-min intervals, we used dataloggers (Minikin Tie, Ø = 20 mm, www.emsbrno.cz) installed close to the banks from 29 June to 11 October 2021. During this period, we collected data relating to the daily discharge levels of stocked streams from the Czech Hydrometeorological Institute (CHMI). The weight and length of all collected fish were measured; temperature data loggers were removed from the water and their data extracted.

Data analysis. For the purposed of this study, we designed the site-specific discharge severity index (D_s) to reflect the discharge severity observed in particular streams during the monitoring period (Suppl. material 1: fig. S1). This index was calculated based on discharge components, the number of peak occurrences, the peak magnitude, and stream slope (Suppl. material 1: tables S1–S4). The equation below represents the final step of D_s calculation by summing site-specific weighted conditions (WC$_i$)

$$D_s = \sum \mathrm{WC}_i$$

A higher D_s number reveals the existence of a more severe discharge; regime; the resulting D_s values were used

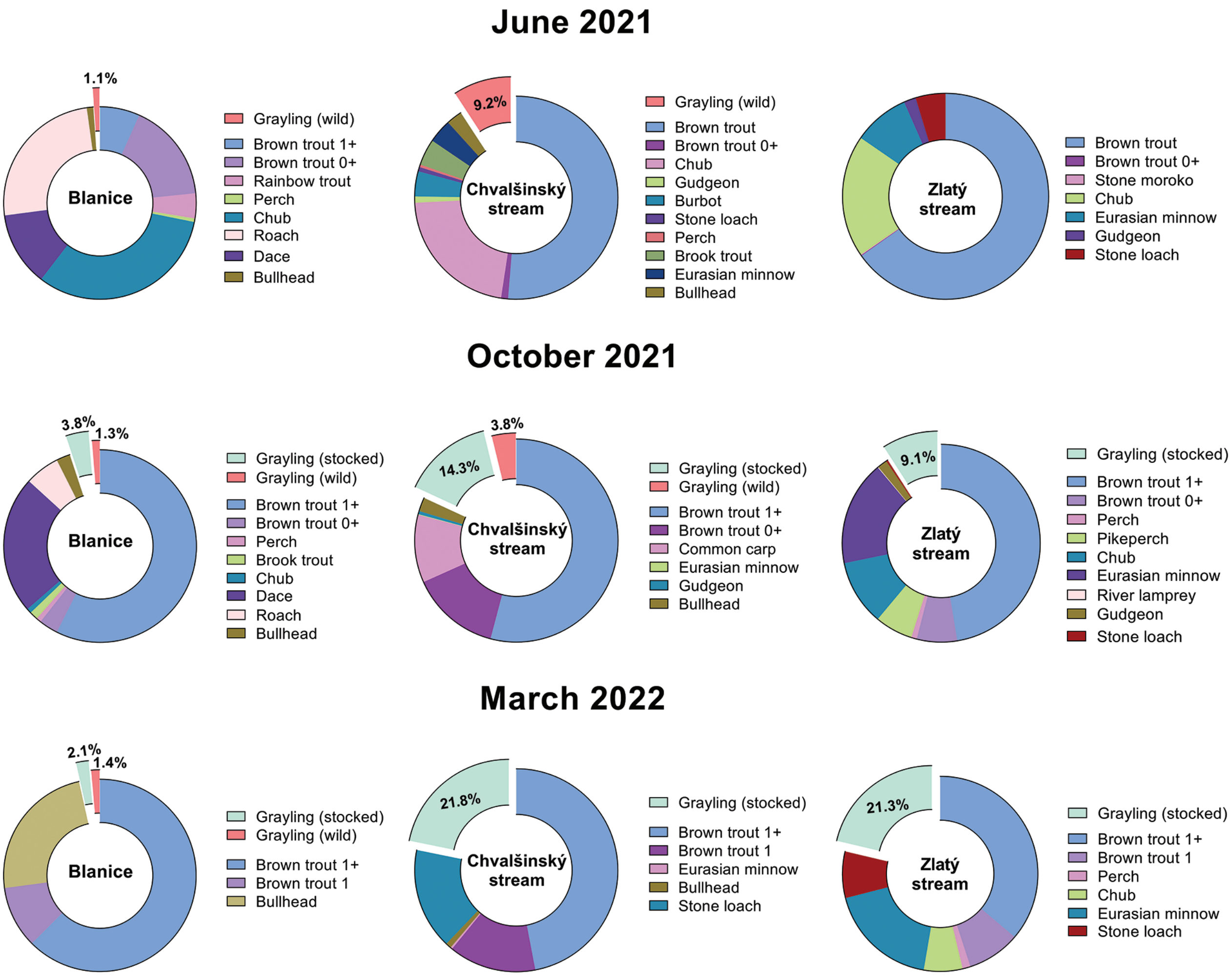

Figure 2. The changes in fish communities at the stocking sites after the three electrofishing monitoring sessions in three streams in the Czech Republic. Values represent biomass [$g \cdot m^{-2}$] expressed in percentages. The pie graphs depict the fish communities recorded in June prior to stocking (top), in October at the end of the growing season (middle), and as overwintering communities in March (bottom).

subsequently in the principal component analysis (PCA). By summing all the measured points extracted from temperature dataloggers, we calculated the time exposure (in hours) of stocked fry to temperatures (range 4–21°C) during the whole of the experiment (Suppl. material 1: fig. S2). We calculated the diel temperature amplitude (T_a) of every stream to compare differences between the maximum and minimum diel temperature values throughout the monitoring period. We recorded the total recapture rates which includes fish recaptured 3.5 months after stocking, both at the release site and within 300 m downstream in the river, relative to the total number stocked. Then, site fidelity of stocked fish, which relates to the fish being recaptured at the initial release site at the end of the growing seasons (October) and in the period from October to spring (March). We also calculated displacement by comparing the number of fish recaptured at a site versus fish recaptured in a 300-m downstream section. Lastly, we calculated overwinter recapture rate refers to fish recaptured at the release site after the winter (March), compared to those detected in same place in a previous electrofishing event (October).

The statistical analysis was performed with the software GraphPad Prism (version 9.5.0). A chi-squared test was used to assess differences in total recapture rates, site fidelity, displacement, and overwintering between the three stocked stretches by looking at stocked grayling recapture rates. A one-way ANOVA followed by a post-hoc Tukey's test was used to assess the differences between the three stocking groups taking into account measured biometric parameters (length, weight, and condition factor) and also for differences in temperature amplitude levels. The three datasets of measured stream temperatures did not pass the D'Agostino-Pearson (omnibus K2) normality test so we used the Kruskal–Wallis non-parametric test with Dunn's multiple comparisons test to assess the differences in temperature between the three sites. Accepted significant differences for values were $P < 0.05$. A cluster analysis (PCA) was conducted using the seven variables to demonstrate the relation between the stocking sites. The variables originating from the monitoring conducted in October used in the PCA were recapture (RC), standard length (SL), condition factor (K), displacement (DP), mean temperature (T_m), discharge severity index (D_s), and diel temperature amplitude (T_a).

Ethical statement. This study was conducted following the ethical guidelines of the Czech Republic and received approval from the relevant ethics committee. The treatment and welfare of fish fully adhered to the legal requirements in the Czech Republic (§ 7 Law No. 114/1992 on The Protection of Nature and Landscape and § 6, 7, 9, and 10 Regulation No. 419/2012 on the Care, Breeding, and Use of Experimental Animals).

Results

The electrofishing survey conducted in October revealed differences in site fidelity and displacement between streams when looking at recapture rates of stocked grayling (Table 2). In March, recapture rates decreased from October at every site and varied between streams.

The tested standard lengths and weights increased significantly from the initial state up to October, with both parameters for the Chvalšinský stream group being significantly higher than the groups from the other two streams (Table 3). The significant standard length and weight increase is presented in Fig. 3. The comparisons of condition factors in the recaptured autumn group, showed increased values for Chvalšinský ($P < 0.001$) and Zlatý stream ($P < 0{,}032$), while the K value significantly decreased in the Blanice fish group ($P < 0.001$). The recaptured group of Chvalšinský exhibited higher K values compared to both Blanice ($P < 0.001$) and Zlatý ($P < 0.001$) groups.

High discharge events mainly occurred in the first month after stocking (Suppl. material 1: fig. S1). The mean discharge was 0.74 $m^3 \cdot s^{-1}$ (SD ± 0.59) for Chvalšinský, 1.35 $m^3 \cdot s^{-1}$ (SD ± 1.02) for Blanice and 0.28 $m^3 \cdot s^{-1}$ (SD ± 0.25) for Zlatý stream, with calculated discharge severity indices (D_s) of 36.4, 47.0 and 17.5, respectively (Suppl. material 1: tables S–S4). Low discharge levels did not last for long during the monitoring period. Comparing the timing of discharge peaks and corresponding stream temperature, we found no signs of thermal discharge peaking or any connection between discharge peak events and alterations in water temperature.

The analyzed stream temperatures (Kruskal–Wallis, Dunn's post-test) revealed that the Blanice stream was the coldest (mean ± SD, 13.9 ± 2.6°C, $P < 0.001$), followed by Chvalšinský (14.1 ± 2.4°C) and Zlatý (14.5 ± 2.5°C). We found that Blanice had a significantly greater diel temperature amplitude (± 2.8°C) than the other two sites. The same comparisons revealed no significant differences between Chvalšinský (± 2.0°C) and Zlatý (± 1.9°C); additionally, Chvalšinský had a narrower temperature range than the other two (Suppl. material 1: fig. S2).

The PCA depicts the cluster separation of recaptured grayling 3.5 months after stocking. We selected the first two principal components (PCs) based on Kaiser's rule (<1 eigenvalue), which described 82.81% of the variance. PC1 described 54.28% and PC2 28.53%. Loadings for principal components show the correlation between the variables and PCs (Table 4). The variables located on the same side of the plot are positively correlated with each other but negatively correlated with variables on the opposite side of the plot. The variables DP, T_a, and D_s with negative loadings are all strongly associated with PC1, but are only very weakly-to-moderately correlated with PC2. Their high negative loadings are significant contributors to PC1 and place them close to the Blanice cluster, which underlines the high values of these variables in the Blanice recapture group. T_m is positioned close to the Zlatý stream cluster and strongly negatively correlates with DP. Conversely, variables SL and RC have high negative loadings and contribute strongly to PC2. They are positioned close to the Chvalšinský stream group, revealing the high values for this cluster. Lastly, the variable T_a shows a strong negative correlation with K, while D_s exhibits a negative correlation with K, albeit to a slightly lesser extent.

Table 2. Total recapture rates, site fidelity, displacement rates, and overwinter recapture rates of stocked advanced fry of the European grayling, *Thymallus thymallus*, in three streams in the Czech Republic.

Stream	Total number of fish stocked	Total recapture rate [%]	Site fidelity [%]	Displacement rate [%]	Overwinter recapture rate [%]
Chvalšinský	1800	4.2^{a}	3.7^{a}	13.2^{a}	13.6^{a}
Blanice	1800	2.5^{b}	1.7^{b}	33.3^{b}	6.7^{b}
Zlatý	1800	1.5^{c}	1.3^{b}	14.8^{c}	78.0^{b}

Superscript letters following the values represent statistical differences between recaptured groups (Chi-square test, $P < 0.05$).

Table 3. Complete biometric data of recaptured stocked fry of the European grayling, *Thymallus thymallus*, in three streams in the Czech Republic, obtained in October 2021 at stocking sites 3.5 months after stocking (autumn recapture) and, in 300 m of the river downstream from the stocking site (displacement), and at the stocking sites in March for overwintering monitoring (March recapture).

Stocking site	Site recapture				Displacement				Overwintering			
	n	SL [cm]	*W* [g]	*K*	*n*	SL [cm]	*W* [g]	*K*	*n*	SL [cm]	*W* [g]	*K*
Chvalšinský	66	13.7 ± 1.0^{a}	34.5 ± 0.8^{a}	0.89 ± 0.09^{a}	10	11.95 ± 0.67	24.6 ± 3.5	1.39 ± 0.03	9	15.18 ± 0.35	48.6 ± 3.3	0.93 ± 0.07
Blanice	30	11.5 ± 0.6^{b}	19.1 ± 0.6^{b}	0.78 ± 0.07^{b}	15	10.58 ± 0.17	14.9 ± 0.7	1.25 ± 0.02	2	12.23 ± 0.64	22.4 ± 3.1	0.78 ± 0.07
Zlatý	23	11.4 ± 0.7^{b}	20.5 ± 0.8^{b}	0.82 ± 0.05^{b}	4	11.30 ± 0.25	17.5 ± 1.4	1.21 ± 0.07	18	13.58 ± 0.29	33.3 ± 2.1	0.86 ± 0.07

n = number of fish, SL = standard length, *W* = weight, *K* = condition factor. The values are mean ± standard deviation); superscript letters following the values represent statistical differences between recaptured groups (Tukey post-hoc test, $P < 0.05$).

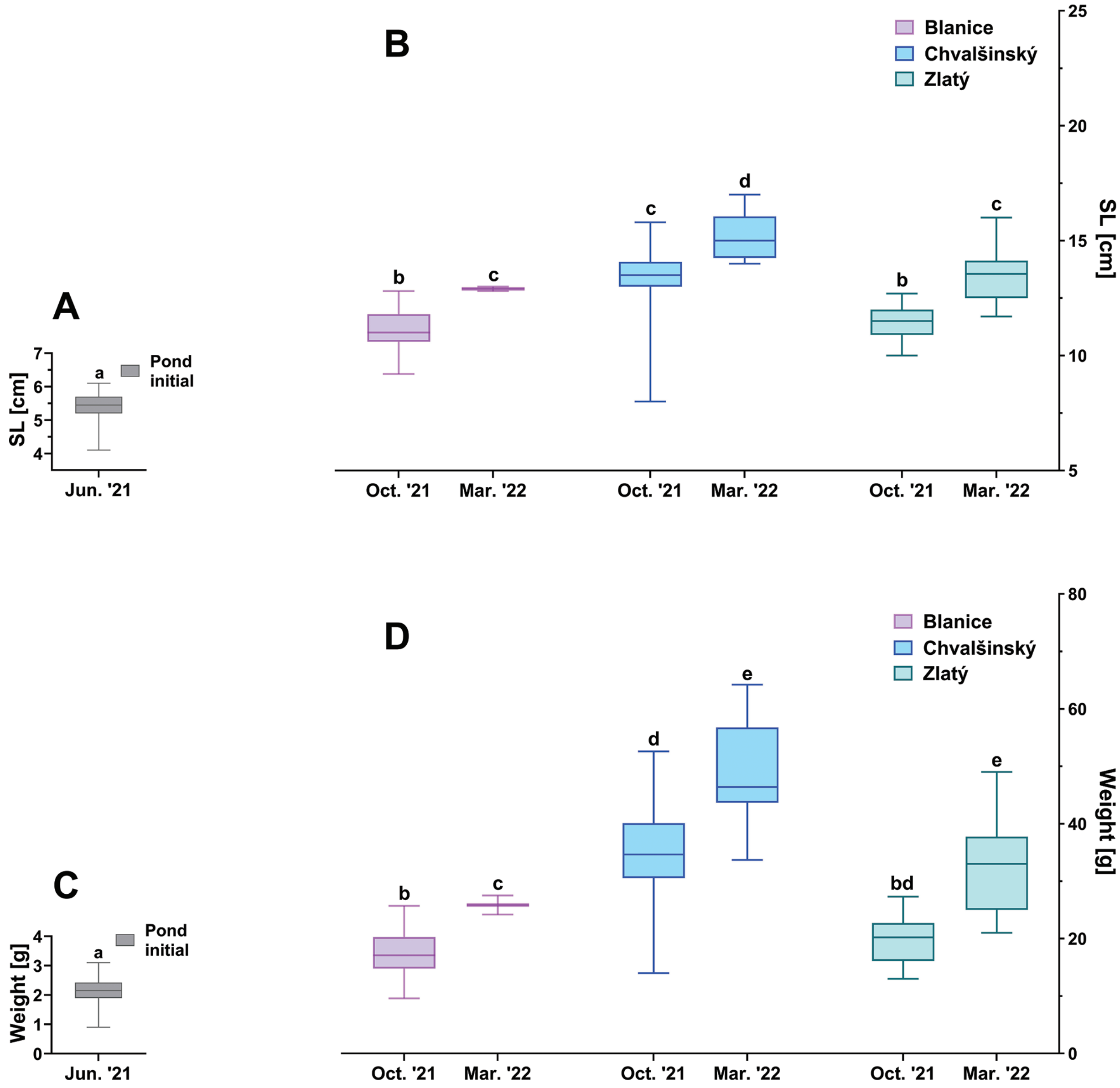

Figure 3. Significant standard length and weight growth in stocked European grayling, *Thymallus thymallus*, from their pre-stocking state (**A**, **C**) in June (2021) and after recapture events (**B**, **D**) in three streams in the Czech Republic in October (2021) and March (2022).

Discussion

The summer mass-stocking performed with advanced fry European grayling notably strengthened the wild grayling population at all three stocked sites. Recorded post-release biomasses 3.5 months after release and after the first winter (Fig. 2) demonstrated an increased presence of EG in each stream. Advanced fry exhibited a substantial increase in weight and length, which varied between the stocked streams, thus reflecting the differences in their habitats. In this study, ARS was justified as a reliable medium for successfully marking young fish stages (Hühn et al. 2014) and a non-invasive detection method (Turek et al. 2024). It was successfully applied for the first time on 0+ EG and fulfilled the needs of the monitoring restocking program for quick ARS trace identification on tail-fin rays, thereby ensuring continuous workflow in the field.

Table 4. The obtained loadings of the variables used in the cluster analysis.

Variable	PC1	PC2
T_a	–0.89	0.42
T_m	0.97	0.24
DP	–1.00	–0.07
RC	–0.13	–0.94
SL	0.22	–0.84
K	0.24	–0.35
D_s	–0.98	–0.21

T_a = diel amplitude in water temperature, T_m = mean temperature, DP = displacement, RC = recapture, SL = standard length, K = condition factor, D_s = discharge severity index.

The increase in pond water temperature (~23°C) signaled the end of the rearing program because, in salmonids at this temperature, growth is halted (Crisp 1996;

Hartman and Jensen 2017) and approaches to the sublethal temperatures which depresses feeding rates (Meeuwig et al. 2004). In pond conditions, temperature-induced mortality along with disease in EG can occur at 17.2°C (Carlstein 1993). However, in the warmer pond water, we detected a good final survivability (27%) followed by the high condition factor of fry fed on zooplankton (Table 1). It confirms that EG fry are tolerant to a steady temperature increase in rearing conditions (Szmyt et al. 2013). The additional advantage of high temperatures in the pond is that they are thought to contribute to the uprising of a critical thermal maximum in grayling (Lohr et al. 1996), thus preventing temperature-induced mortality in the wild.

Post-stocking biometric parameters recorded after 3.5 months suggest satisfactory adaptation by stocked fry (Table 2), with the only drop in K compared to the initial state occurring in the Blanice group. This confirms the adaptive potential of the 0+ age category after being fed on natural food (Carlstein 1997; Czerniawski 2015). Recaptured fish exhibited rapid growth after stocking in all three stretches, with more than a twofold increase in standard length during the growing season in their natural habitat. This growth pattern closely resembles the developmental dynamics observed in young, wild conspecifics (Persat and Pattee 1981). Therefore, we conclude that recaptured survivors were able to utilize the favorable stream conditions during the growing season, as shown by their mean length that exceeded even that of intensively tank-reared 0+ EG harvested during the same period (Carlstein 1993).

Our results show that extended exposure to colder water leads to reduced biometrics (Blanice fish group), while warmer streams provide longer growth-favorable temperatures and better biometrics. We conceived the timing of the stocking to coincide with the growth-favorable conditions in summer including high prey abundance for grayling (Thorfve and Carlstein 1998) and higher water temperatures (Deegan et al. 1999; Mallet et al. 1999; Marsh et al. 2022). Accordingly, in July at the beginning of the adaptation to the riverine environment 0+ grayling faced little dietary overlap with 0+ brown trout (Degerman et al. 2000). Therefore, the timing was probably suitable for preventing poor nutrition and starvation, which could cause exhaustion and subsequent mortality amongst the stocked fish (Bachman 1984). Cold water can be a stronger growth-limiting factor in juvenile salmonids than limitations on feeding (Nicieza and Metcalfe 1997). However, colder water can significantly decrease feeding efficiency in reared grayling at 5°C (Watz et al. 2014). Additionally, this stretch of Blanice is further from sources of fertilizers—which could lead to smaller prey organisms (Deegan et al. 1999)—than the two other streams that are near upstream towns (Chvalšinský) or agricultural fields (Zlatý). Conversely, there were also no growth-limiting temperature ranges in the Chvalšinský stream during the growing season, as reflected in the higher biometrics at this site (Table 3).

Recorded displacement in downstream stretches also varied and was strongly associated with the severity of natural discharge regimes, cold water, and the high diel temperature amplitude (Fig. 4). Stocked fry recaptured at Blanice experienced the highest D_s index, with one flood-like event during the growing season (Fig. 4) that led to the highest level of displacement. This confirmed the inability of 0+ grayling to withstand this kind of impact and resulted in increased drift (Auer et al. 2023) as occurs in other young salmonids (Jowett and Richardson 1989; Hayes et al. 2010; Warren et al. 2015). Accordingly, the domestication of salmonids can induce pronounced morphological changes in body shape as a response to the environment (Pulcini et al. 2013; Bajić et al. 2018) and reduce swimming abilities (Reinbold et al. 2009). Therefore, it is not reasonable to expect high post-release site fidelity of 0+ stocked grayling in steep streams with demanding natural discharge. These conditions also imply higher energy demands (Heggenes and Traaen 1988) and reduce the growth in 0+ EG (Marsh et al. 2022), which might explain the low biometry in the Blanice stocked group compared to the two other groups.

We did not find any clear natural discharge thermal peaking patterns at the study sites. However, we showed that the most pronounced disappearance of 0+ grayling up to March occurred in the coldest stocking stream (Blanice), which had the highest diel T_a and $D_{s.}$ In addition, the Blanice site was the shallowest and the disappearance could have been prompted by a lack of thermally stratified deep pools, whose presence is important for juvenile salmonids (Nielsen et al. 1994). Coupled with the high diel T_a, this lack of pools may have forced fish to seek appropriate thermal habitats (Fausch and Bramblett 1991; Hartman and Jensen 2017), resulting in higher displacement. The same deeper pools could provide refuge for the fry, creating microhabitats with lower water velocity (Fausch and Bramblett 1991).

The lowest total recapture rate during the growing season was at Zlatý, which was characterized by the highest mean temperatures. Even so, based on the literature these temperature ranges cannot be regarded as either sublethal or lethal. However, they may have caused a steep decrease in juvenile European grayling recruitment (Charles et al. 2006), which could explain our results. This site had the lowest D_s, and stranding was supposed not to be pronounced in streams with steeper banks (Tuhtan et al. 2012). The disappearance from the release site on the Zlatý was more likely driven by strong density-dependent displacement due to the small habitat capacity that would have reduced competition. It is known that intraspecific density affects 0+ EG recruitment (Bašić et al. 2018), while habitat spatial limitation is thought to lead to displacement caused by population self-thinning (Hayes et al. 2010). An additional aspect could be the presence of the well-developed riparian canopy along this stretch, which might negatively affect the survival of small grayling during the growing season (Marsh et al. 2021).

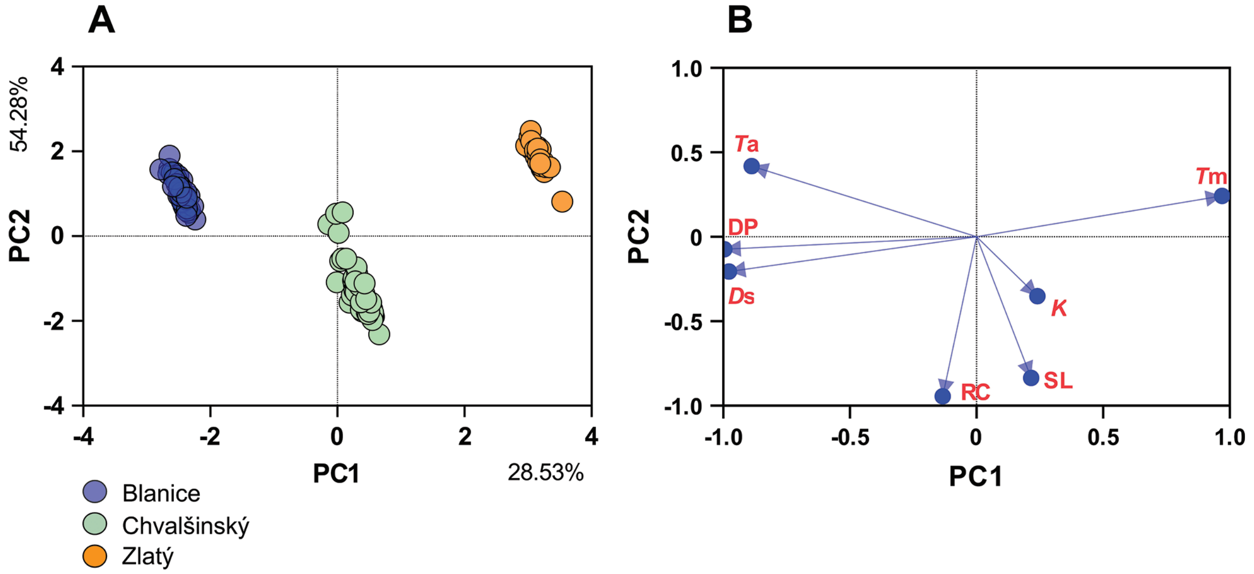

Figure 4. PCA score (**A**) and loading (**B**) plots of European grayling, *Thymallus thymallus*, in three streams in the Czech Republic. On the scores plot, individual fish are represented by a single dot. Explanation of variables: RC = number of recaptured fish 3.5 months after stocking at the stocking site; SL = standard length; K = condition factor; D_s = discharge severity index; DP = number of fish recaptured in the downstream stretch; T_m = mean temperature for the monitored period; T_a = the level of diel variation between maximal and minimal temperature values.

Our study provides evidence that the first winter creates strong selective pressure on fish communities and can significantly reduce fish biomasses (Suppl. material 1: tables S5–S7). In our study it increased the disappearance of 0+ stocked grayling, especially at Blanice (autumn/spring = 30/2), thereby confirming the high magnitude of overwintering loss in young stocked salmonids (Biro et al. 2021). Despite this, the proportion of stocked grayling fry at the release sites did substantially increase in comparison with autumn in two of the streams, Chvalšinský and Zlatý. Furthermore, recaptured fish in October to March significantly grew (SL) at every site (Table 3), confirming that autumn and winter still can provide growth-supportive conditions for grayling 0+, although not nearly as much as reported for 1+ and 2+ grayling (Marsh et al. 2022). The highest biometrics in the Chvalšinský group did not correspond with high overwinter recapture (winter loss 86%), which implies that local habitat characteristics can dictate the extent of overwinter mortality (Hurst 2007). It seems that the thick canopy of riparian vegetation at Zlatý and its submerged parts favored both overwinter survival and site fidelity in this site (78%). This implies that such streams could serve as valuable refuges for the desirable long-term adaptation of stocked grayling fry over the first winter and, potentially, at the start of the growing season these individuals can be longitudinally translocated within the water body.

In this stocking region, where fish predators are abundant and winter freezing affects ponds, predation is crucial in determining mortality in small streams. Accordingly, herons can be attracted by increased densities after stocking and become the main fish predators in shallow water habitats (Miyamoto et al. 2017). Given that reared salmonids suffer from heron predation (Petersson and Järvi 2006), the availability of appropriate shelters such as riparian canopy trees and submerged vegetation may have mitigated the impact of this type of predation.

In this stocking experiment, advanced grayling fry (5–6 cm) were released in the middle of the growing season to ensure appropriate stocking size and timing, reducing post-stocking losses. It was shown that this length of grayling fry has already demonstrated enhanced swimming abilities that allows better habitat use than the smaller conspecifics (Bardonnet et al. 1991). Conversely, using younger grayling fry, such as sac fry (2–2.5 cm), for stocking could make them vulnerable to unfavorable discharges (Poff et al. 1997; Auer et al. 2017) and lead to massive post-stocking displacement (Valentin et al. 1994).

Release sites with heterogeneous habitats, rich instream shelters, and thermal and flood refuge zones were considered to ensure the viability of stocked grayling. Evidently, stretches with steep gradients with frequent flood-like discharges should be avoided as stocking sites for 0+ grayling, with careful consideration of stocking size being crucial, given their significant impact on population abundances (Jowett and Richardson 1989; Hayes et al. 2010) and even lead to subsequent local extinctions (Warren et al. 2015). In the hydropeaking rivers that negatively impact 0+ grayling (Auer et al. 2017), our results imply that effective stocking actions should be performed farther from hydropower plants due to hydropeaking events whose effects tend to decrease downstream (Greimel et al. 2018). Finally, the stocking outcomes for grayling fry depend on stochastic natural events, which implies the need for stocking actions to be repeated to match the most favorable seasonal conditions and support their recruitment.

Besides serving as an example of how stocking practices can be evaluated, this study could be a starting point in enlightening the reasons behind the stocking failures with EG. We suggest further research to focus on the seasonal comparison between post-stocked grayling and wild conspecifics, e.g. their growth and survival rates, age of maturation, and reproduction potential (Rulifson and Laney 1999), to direct future management actions.

Conclusion

This stocking program study describes a perspective approach for the long-term enhancement of wild European grayling populations. The rearing technique was suitable for rearing large numbers of grayling fry with good adaptability to the wild environment. For identifying young EG, we recommend using ARS as a non-invasive detection procedure, particularly when assessing restocking programs aimed at conserving endangered species.

Acknowledgments

The research was supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project PROFISH (CZ.02.1.01/0.0/0.0/16_019/0000869) and by the Ministry of Agriculture of the Czech Republic – project of National Agency for Agricultural Research (NAZV) No. QK23020064.

References

Auer S, Zeiringer B, Führer S, Tonolla D, Schmutz S (2017) Effects of river bank heterogeneity and time of day on drift and stranding of juvenile European grayling (*Thymallus thymallus* L.) caused by hydropeaking. Science of the Total Environment 575: 1515–1521. https://doi.org/10.1016/j.scitotenv.2016.10.029

Auer S, Hayes DS, Führer S, Zeiringer B, Schmutz S (2023) Effects of cold and warm thermopeaking on drift and stranding of juvenile European grayling (*Thymallus thymallus*). River Research and Applications 39(3): 401–411. https://doi.org/10.1002/rra.4077

Avramović M, Turek J, Tomčala A, Mráz J, Bláha M, Let M, Szmyt M, Randák T (2024) Assessing the acclimatisation to the wild of stocked European graylings *Thymallus thymallus* by monitoring lipid dynamics and food consumption. Knowledge and Management of Aquatic Ecosystems 2024(425): e10. https://doi.org/10.1051/kmae/2024008

Bachman RA (1984) Foraging behavior of free-ranging wild and hatchery brown trout in a stream. Transactions of the American Fisheries Society 113(1): 1–32. https://doi.org/10.1577/1548-8659(1984)113<1:FBOFWA>2.0.CO;2

Bajić A, Jojić V, Snoj A, Miljanović B, Askeyev O, Askeyev I, Marić S (2018) Comparative body shape variation of the European grayling *Thymallus thymallus* (Actinopterygii, Salmonidae) from wild populations and hatcheries. Zoologischer Anzeiger 272: 73–80. https://doi.org/10.1016/j.jcz.2017.12.005

Bardonnet A, Gaudin P, Persat H (1991) Microhabitats and diel downstream migration of young grayling (*Thymallus thymallus* L.). Freshwater Biology 26: 365–376. https://doi.org/10.1111/j.1365-2427.1991.tb01404.x

Bašić T, Britton JR, Cove RJ, Ibbotson AT, Gregory SD (2018) Roles of discharge and temperature in recruitment of a cold-water fish, the European grayling *Thymallus thymallus*, near its southern range limit. Ecology Freshwater Fish 27(4): 940–951. https://doi.org/10.1111/eff.12405

Beauregard D, Enders E, Boisclair D (2013) Consequences of circadian fluctuations in water temperature on the standard metabolic rate of Atlantic salmon parr (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 70(7): 1072–1081. https://doi.org/10.1139/cjfas-2012-0342

Biro PA, Post JR, Beckmann C (2021) Autumn lipid reserves, overwinter lipid depletion, and high winter mortality of rainbow trout in experimental lakes. Canadian Journal of Fisheries and Aquatic Sciences 78(6): 738–743. https://doi.org/10.1139/cjfas-2020-0276

Brignone S, De Santis V, Putelli T, Molina C, Piccinini A, Carmichael RA, Volta P (2022) What's the effectiveness of stocking actions in small creeks? The role of water discharge behind hatchery trout downstream movement. PeerJ 10: e14069. https://doi.org/10.7717/peerj.14069

Carlstein M (1993) Natural food and artificial, dry starter diets: Effects on growth and survival in intensively reared European grayling. Aquaculture International 1(2): 112–123. https://doi.org/10.1007/BF00692615

Carlstein M (1997) Effects of rearing technique and fish size on post-stocking feeding, growth and survival of European grayling, *Thymallus thymallus* (L.). Fisheries Management and Ecology 4(5): 391–404. https://doi.org/10.1046/j.1365-2400.1997.00068.x

Charles S, Mallet J-P, Persat H (2006) Population dynamics of grayling: Modelling temperature and discharge effects. Mathematical Modelling of Natural Phenomena 1(1): 31–48. https://doi.org/10.1051/mmnp:2006002

Crisp DT (1996) Environmental requirements of common riverine European salmonid fish species in fresh water with particular reference to physical and chemical aspects. Hydrobiologia 323(3): 201–221. https://doi.org/10.1007/BF00007847

Czerniawski R, Domagala J, Krepski T, Pilecka-Rapacz M (2015) The effect of the live diet given to hatchery-reared fry of the European grayling (*Thymallus thymallus*) on their survival and growth in the wild. Turkish Journal of Fisheries and Aquatic Sciences 15(3): 633–638. https://doi.org/10.4194/1303-2712-v15_3_07

Deegan LA, Golden HE, Harvey CJ, Peterson BJ (1999) Influence of environmental variability on the growth of age-0 and adult Arctic grayling. Transactions of the American Fisheries Society 128(6): 1163–1175. https://doi.org/10.1577/1548-8659(1999)128<1163:IOEVOT>2.0.CO;2

Degerman E, Näslund I, Sers B (2000) Stream habitat use and diet of juvenile (0+) brown trout and grayling in sympatry. Ecology Freshwater Fish 9(4): 191–201. https://doi.org/10.1111/j.1600-0633.2000.eff090401.x

Enders EC, Stickler M, Pennell CJ, Cote D, Alfredsen K, Scruton DA (2008) Variations in distribution and mobility of Atlantic salmon parr during winter in a small, steep river. Hydrobiologia 609(1): 37–44. https://doi.org/10.1007/s10750-008-9401-5

Fausch KD, Bramblett RG (1991) Disturbance and fish communities in intermittent tributaries of a western great plains river. Copeia 1991(3): 659–674. https://doi.org/10.2307/1446392

Fraser D, Weir L, Bernatchez L, Hansen MM, Taylor EB (2011) Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. Heredity 106: 404–420. https://doi.org/10.1038/hdy.2010.167

Greimel F, Schülting L, Graf W, Bondar-Kunze E, Auer S, Zeiringer B, Hauer C (2018) Hydropeaking impacts and mitigation. Pp. 91–110. In: Schmutz S, Sendzimir J (Eds.) Riverine ecosystem management: Science for governing towards a sustainable future. Springer International Publishing, Cham, Switzerland. https://doi.org/10.1007/978-3-319-73250-3_5

Halačka K, Poštulková E, Kopp R, Mareš J, Vetešník L (2018) Možnosti značení vysazovaných ryb pomocí ARS – aplikace a detekce. Pp. 37–43. In: Kopp R, Mareš J, Grmela J (Eds.) Účelové rybí obsádky na vodárenských nádržích. Brno, Czech Republic, 11 October 2018.

Hartman KJ, Jensen OP (2017) Anticipating climate change impacts on Mongolian salmonids: Bioenergetics models for lenok and Baikal grayling. Ecology Freshwater Fish 26(3): 383–396. https://doi.org/10.1111/eff.12282

Hayes JW, Olsen DA, Hay J (2010) The influence of natural variation in discharge on juvenile brown trout population dynamics in a nursery tributary of the Motueka River, New Zealand. New Zealand Journal of Marine and Freshwater Research 44(4): 247–269. https://doi.org/10.1080/00288330.2010.509905

Hayes HDS, Lautsch E, Unfer G, Greimel F, Zeiringer B, Höller N, Schmutz S (2021) Response of European grayling, *Thymallus thymallus*, to multiple stressors in hydropeaking rivers. Journal of Environmental Management 292: 112737. https://doi.org/10.1016/j.jenvman.2021.112737

Heggenes J, Traaen T (1988) Downstream migration and critical water velocities in stream channels for fry of four salmonid species. Journal of Fish Biology 32(5): 717–727. https://doi.org/10.1111/j.1095-8649.1988.tb05412.x

Horká P, Horký P, Randák T, Turek J, Rylková K, Slavík O (2015) Radio-telemetry shows differences in the behaviour of wild and hatchery-reared European grayling *Thymallus thymallus* in response to environmental variables. Journal of Fish Biology 86(2): 544–557. https://doi.org/10.1111/jfb.12575

Hühn D, Lübke K, Skov C, Arlinghaus R (2014) Natural recruitment, density-dependent juvenile survival, and the potential for additive effects of stock enhancement: An experimental evaluation of stocking northern pike (*Esox lucius*) fry. Canadian Journal of Fisheries and Aquatic Sciences 71(10): 1508–1519. https://doi.org/10.1139/cjfas-2013-0636

Hurst TP (2007) Causes and consequences of winter mortality in fishes. Journal of Fish Biology 71(2): 315–345. https://doi.org/10.1111/j.1095-8649.2007.01596.x

Janković D (1964) Synopsis of biological data on European grayling (*Thymallus thymallus* (Linnaeus) 1758). FAO Fisheries Synopsis, No. 24, F1. FAO, Rome, 50 pp.

Jepsen N, Ravn HD, Pedersen S (2018) Change of foraging behavior of cormorants and the effect on river fish. Hydrobiologia 820(1): 189–199. https://doi.org/10.1007/s10750-018-3656-2

Jowett IG, Richardson J (1989) Effects of a severe flood on instream habitat and trout populations in seven New Zealand rivers. New Zealand Journal of Marine and Freshwater Research 23(1): 11–17. https://doi.org/10.1080/00288330.1989.9516335

Lohr SC, Byorth PA, Kaya CM, Dwyer WP (1996) High-temperature tolerances of fluvial Arctic grayling and comparisons with summer river temperatures of the big Hole River, Montana. Transactions of the American Fisheries Society 125(6): 933–939. https://doi.org/10.1577/1548-8659(1996)125<0933:HTTOFA>2.3.CO;2

Lorenzen K (2005) Population dynamics and potential of fisheries stock enhancement: Practical theory for assessment and policy analysis. Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences 360(1453): 171–189. https://doi.org/10.1098/rstb.2004.1570

Lorenzen K, Camp EV (2019) Density-dependence in the life history of fishes: When is a fish recruited? Fisheries Research 217: 5–10. https://doi.org/10.1016/j.fishres.2018.09.024

Luczynski M, Zaporowski RR, Golonka JS (1986) Rearing of European grayling, *Thymallus thymallus* L., larvae using dry and live food. Aquaculture Research 17(4): 275–280. https://doi.org/10.1111/j.1365-2109.1986.tb00114.x

Malcolm IA, Soulsby C, Hannah DM, Bacon PJ, Youngson AF, Tetzlaff D (2008) The influence of riparian woodland on stream temperatures: Implications for the performance of juvenile salmonids. Hydrological Processes 22(7): 968–979. https://doi.org/10.1002/hyp.6996

Mallet JP, Charles S, Persat H, Auger P (1999) Growth modelling in accordance with daily water temperature in European grayling (*Thymallus thymallus* L.). Canadian Journal of Fisheries and Aquatic Sciences 56(6): 994–1000. https://doi.org/10.1139/f99-031

Marsh JE, Cove RJ, Britton JR, Wellard RG, House A, Gregory SD (2021) Medium-term environmental changes influence age-specific survival estimates in a salmonid population. Freshwater Biology 66(8): 1530–1545. https://doi.org/10.1111/fwb.13736

Marsh JE, Cove RJ, Britton JR, Wellard RG, Bašić T, Gregory SD (2022) Density-dependence and environmental variability have stage-specific influences on European grayling growth. Oecologia 199(1): 103–117. https://doi.org/10.1007/s00442-022-05163-2

Meeuwig MH, Dunham JB, Hayes JP, Vinyard GL (2004) Effects of constant and cyclical thermal regimes on growth and feeding of juvenile cutthroat trout of variable sizes. Ecology Freshwater Fish 13(3): 208–216. https://doi.org/10.1111/j.1600-0633.2004.00052.x

Miyamoto K, Squires TE, Araki H (2017) Predation of stocked masu salmon (*Oncorhynchus masou*) by riparian wildlife. Canadian Journal of Fisheries and Aquatic Sciences 14: 127472. https://doi.org/10.1101/127472

Nicieza AG, Metcalfe NB (1997) Growth compensation in juvenile Atlantic salmon: Responses to depressed temperature and food availability. Ecology 78(8): 2385–2400. https://doi.org/10.1890/0012-9658(1997)078[2385:GCIJAS]2.0.CO;2

Nielsen JL, Lisle TE, Ozaki V (1994) Thermally stratified pools and their use by steelhead in northern California streams. Transactions of the American Fisheries Society 123(4): 613–626. https://doi.org/10.1577/1548-8659(1994)123<0613:TSPATU>2.3.CO;2

Northcote TG (1995) Comparative biology and management of Arctic and European grayling (Salmonidae, *Thymallus*). Reviews in Fish Biology and Fisheries 5(2): 141–194. https://doi.org/10.1007/BF00179755

Persat H, Pattee E (1981) The growth rate of young grayling in some French rivers. SIL Proceedings, 1922–2010 21: 1270–1275. https://doi.org/10.1080/03680770.1980.11897171

Petersson E, Järvi T (2006) Anti-predator response in wild and sea-ranched brown trout and their crosses. Aquaculture 253(1–4): 218–228. https://doi.org/10.1016/j.aquaculture.2005.08.012

Poff N, Allan JD, Bain M, Karr J, Prestegaard K, Richter B, Sparks R, Stromberg J (1997) The Natural Flow Regime. Bioscience 47(11): 769–784. https://doi.org/10.2307/1313099

Pulcini D, Wheeler PA, Cataudella S, Russo T, Thorgaard GH (2013) Domestication shapes morphology in rainbow trout *Oncorhynchus mykiss*. Journal of Fish Biology 82(2): 390–407. https://doi.org/10.1111/jfb.12002

Reinbold D, Thorgaard GH, Carter PA (2009) Reduced swimming performance and increased growth in domesticated rainbow trout, *Oncorhynchus mykiss*. Canadian Journal of Fisheries and Aquatic Sciences 66(7): 1025–1032. https://doi.org/10.1139/F09-064

Rulifson R, Laney W (1999) Striped bass stocking programs in the United States: Ecological and resource management issues. Canadian Stock Assessment Secretariat, Research Document 99/007. Fisheries and Oceans Canada, Ottawa.

Szmyt M, Goryczko K, Grudniewska J, Lejk A, Wisniewska A, Woźniak M (2013) Preliminary results of European grayling (*Thymallus thymallus* L.) fry rearing to the autumn fingerlings stage. Polish Journal of Natural Sciences 28: 471–483.

Thorfve S (2002) Impacts of in-stream acclimatization in post-stocking behaviour of European grayling in a Swedish stream. Fisheries Management and Ecology 9(5): 253–260. https://doi.org/10.1046/j.1365-2400.2002.00305.x

Thorfve S, Carlstein M (1998) Post-stocking behaviour of hatchery-reared European grayling, *Thymallus thymallus* (L.), and brown trout, *Salmo trutta* L., in a semi-natural stream. Fisheries Management and Ecology 5(2): 147–159. https://doi.org/10.1046/j.1365-2400.1998.00087.x

Tuhtan JA, Noack M, Wieprecht S (2012) Estimating stranding risk due to hydropeaking for juvenile European grayling considering river morphology. KSCE Journal of Civil Engineering 16(2): 197–206. https://doi.org/10.1007/s12205-012-0002-5

Turek J, Horký P, Zlábek V, Velisek J, Slavik O, Randák T (2012) Recapture and condition of pond-reared, and hatchery-reared 1+ European grayling stocked in addition to wild conspecifics in a small river. Knowledge and Management of Aquatic Ecosystems 405(405): 10. https://doi.org/10.1051/kmae/2012016

Turek J, Zlábek V, Velíšek J, Lepič P, Červený D, Randák T (2018) Influence of geographic origin on post-stocking survival and condition of European grayling (*Thymallus thymallus*) in a small river. Aquatic Living Resources 31: 29. https://doi.org/10.1051/alr/2018019

Turek J, Lepic P, Bořík A, Galicová P, Nováková P, Avramović M, Randák T (2024) Evaluation of large-scale marking with alizarin red S in different age rainbow trout fry for nonlethal field identification. Acta Ichthyologica et Piscatoria 54: 43–48. https://doi.org/10.3897/aiep.54.117547

Valentin S, Sempeski P, Souchon Y, Gaudin P (1994) Short-term habitat use by young grayling (*Thymallus thymallus* L.) under variable flow conditions in an experimental stream. Fisheries Management and Ecology 1: 57–65. https://doi.org/10.1111/j.1365-2400.1970.tb00006.x

Waples RS, Ford MJ, Schmitt D (2007) Chapter 21; Empirical results of salmon supplementation in the northeast Pacific: A preliminary assessment. Pp. 383–403 In: Bert TM (Ed.) Ecological and genetic implications of aquaculture activities. Vol 6. Methods and Technologies in Fish Biology and Fisheries, Springer, Dordrecht, Netherlands. https://doi.org/10.1007/978-1-4020-6148-6_21

Warren M, Dunbar MJ, Smith C (2015) River flow as a determinant of salmonid distribution and abundance: A review. Environmental Biology of Fishes 98(6): 1695–1717. https://doi.org/10.1007/s10641-015-0376-6

Watz J (2017) Overwintering behaviour of stocked brown trout: effects of the rearing environment and river habitat complexity. In: 50th Anniversary Symposium of the Fisheries Society of the British Isles, 3–7 July 2017, University of Exeter, UK. http://urn.kb.se/resolve?urn=urn:nbn:se:kau:diva-65010

Watz J, Bergman E, Piccolo J, Greenberg L (2014) Prey capture rates of two species of salmonids (*Salmo trutta* and *Thymallus thymallus*) in an artificial stream: Effects of temperature on their functional response. Marine and Freshwater Behaviour and Physiology 47(2): 93–99. https://doi.org/10.1080/10236244.2014.900210

Supplementary material 1

Calculation of the discharge data in order to descrbie the hydrological conditions of three analyzed streams

Authors: Mladen Avramović, Jan Turek, Pavel Lepič, Mariusz Szmyt, Jan Pastejřík, Tomáš Randák

Data type: docx

Explanation note: **table S1.** The overview of discharge levels of monitored stretches with the frequency of detected discharge peaks placed according to their discharge magnitude. The discharge peaks classification starts with two-fold and finishes on the six-fold increase from mean discharge. **table S2.** Discharge conditions of monitored stocking stretches. **table S3.** Slope coefficients of monitored stretches. **tables S4.** The final calculation of the discharge severity indices (D_s) represented by total values for every stream. **table S5.** Fish community data recorded during three recapture events on the Blanice river. **table S6.** Fish community data recorded during three recapture events on the Chvalšinský stream. **table S7.** Fish community data recorded during three recapture events on the Zlatý stream. **fig. S1.** Recorded discharge dynamics of the three stocking stretches after the fish release on 29 June 2021 until recapture on 11 October 2021. Measurements obtained from the nearest CHMI monitoring stations. **fig. S2.** Duration of exposure of stocked European grayling fry in the three studied streams to the observed temperature ranges from 29 June to 11 October 2021.

Link: https://doi.org/10.3897/aiep.54.124289.suppl1