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RESEARCH ARTICLE

Influence of geographic origin on post-stocking survival and condition of European grayling (*Thymallus thymallus*) in a small river

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Abstract – The post-release survival and condition of 1+ year old European grayling *Thymallus thymallus* reared in a local (Husinec) and two 250–300 km distant, hatcheries (Pardubice and Hynčice) were evaluated by the recapture of tagged fish five months after release into the Blanice River, Czech Republic, the fish population of which was depleted by cormorant predation during several previous winters. The fish were marked using Visible Implant Elastomer tags and released into six sites along the river in May 2014: 100 locally sourced fish and 100 of a strain from a distant source per site. Significantly higher recapture of Husinec (33%) than Pardubice (20%) was found at one site but was offset by results in the remaining two sites into which they were stocked. Significantly higher site fidelity was shown by Husinec (12%) than Hynčice (7%) fish in the three sites. Lower initial weight and condition factor of the Husinec fish was equal to or higher than fish from the distant hatcheries at recapture. Some differences in final weight, length, and condition factor were found among groups regardless of release site. Lower recapture rate, growth, and condition were displayed in fish released into the three downstream sites (Husinec 1 and Hynčice). The results indicated the potential for successful use of non-autochthonous stock for grayling population recovery.

Keywords: Population restoration / fishery management / restocking / recapture / European grayling

1 Introduction

European grayling *Thymallus thymallus* L. is a valuable fish species widely distributed in the Central and Northern Europe but is dependent on water of excellent quality with low temperature and high oxygen content. Deterioration of the aquatic environment including low water quality, changes in river morphology and flow regime, spawning grounds destruction, overfishing by sport anglers, and predation have considerably decreased grayling populations in the majority of European waters (Magee, 1993; Northcote, 1995; Uiblein et al., 2001). Despite its status on the European IUCN Red List of Threatened Species as "least concern" (Freyhof, 2011; Freyhof and Brooks, 2011), the status in the Czech Republic is "near threatened" (Lusk et al., 2004), "vulnerable" in France (UICN France et al., 2010) and Austria (Wolfram and Mikschi, 2007), "threatened" in Switzerland (Kirchhöfer et al., 2007), and "endangered" in Germany (Freyhof, 2009). Gum et al. (2006) reported cessation of European grayling stocking programs and a continued relatively low level of captive breeding programmes and support for breeding until ongoing cormorant and goosander predation problems are resolved. There are currently several programs in the Czech Republic aimed at restoring extinct grayling populations (Czech Anglers' Union, personal communication).

To re-establish threatened and extinct populations and expand the recreational fishery, hatchery-reared fish are commonly stocked. The success of stocking fish into natural waters depends on a variety of factors including fish origin, conditioning and acclimatization, handling and transportation, stocking density, and size and age, as well as timing of stocking and predation (Cowx, 1994). Destruction of wild grayling populations, often used as broodfish reservoirs, leads to insufficient local stock, forcing managers of fishing grounds to purchase and stock fish from producers outside the region.

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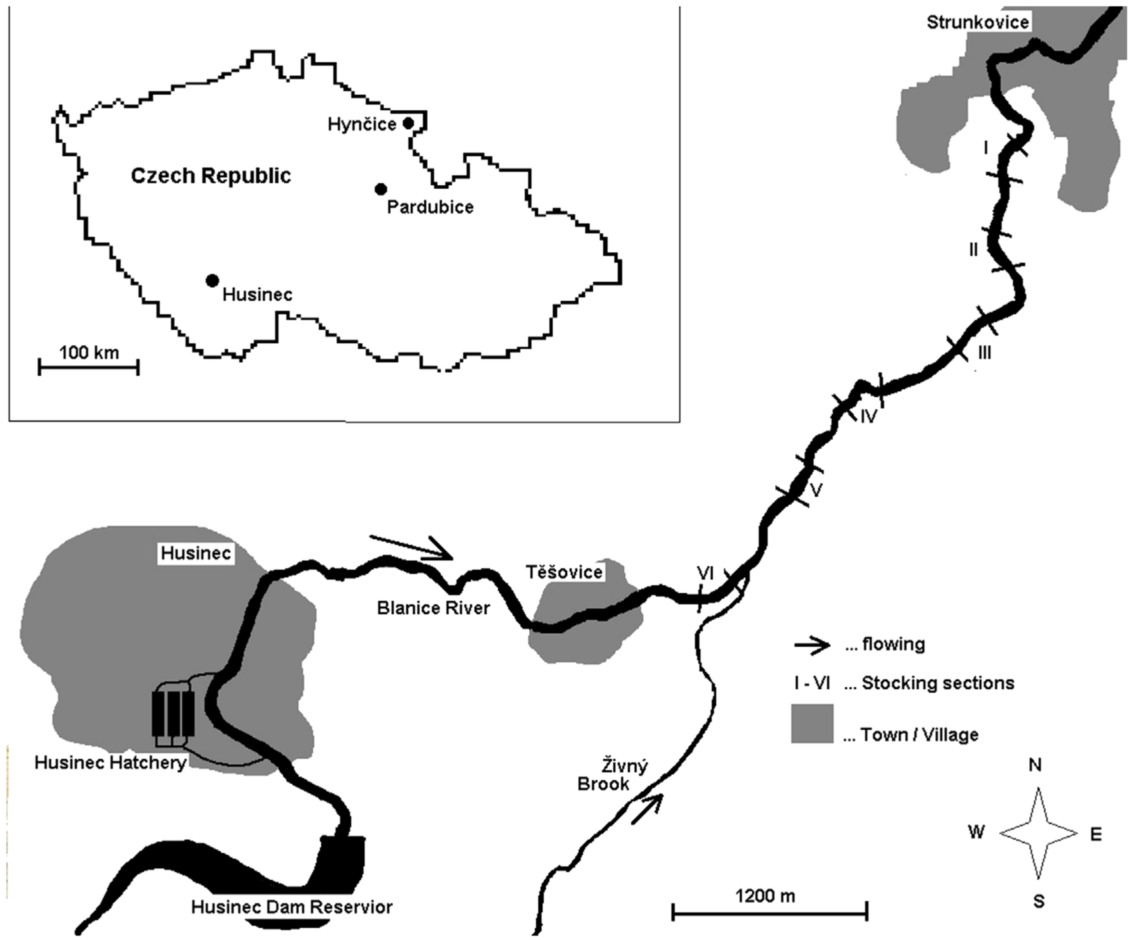

Fig. 1. Map showing the locations of release sites in the Blanice River. The inset shows location hatcheries producing the fish used in the survey.

Some reports indicate problems with non-local fish. Johnsen and Hesthagen (1990) reported higher recapture rates of brown trout stocked into streams near their rearing site than into distant areas. Stress of transportation may result in lower survival, growth, and fecundity of stocked salmonids compared to resident wild fish (Jonsson et al., 1999; Finstad et al., 2003). Although some authors have pointed to the risk of genetic disruption in local grayling populations when stocking trans-basin fish (Meraner et al., 2013; Weiss et al., 2013), the use of extrinsic stock is often the only possibility for re-establishing deteriorating and depleted grayling populations.

The aim of this study was to investigate the effect of geographic origin and transport of fish on their post-stocking performance by assessing recapture rates of hatchery-reared 1+ year-old *T. thymallus* from one local and two distant hatcheries released into the Blanice River in South Bohemia, Czech Republic.

2 Materials and methods

2.1 Study area

The Blanice River (Vltava basin) in South Bohemia is 93 km long, with a drainage area of 860 km^2. Six release sites of 150–190 m were designated in a 5 km stretch of the river (river km 53–58) in a protected area (fishing prohibited) downstream of the Husinec reservoir (37 ha; 2.5×10^6 m^3) (Fig. 1). The Husinec reservoir provides a drinking water supply, flood protection, and hydroelectric power without hydropeaking. Water flow in this stretch of the river is characterized by wide seasonal fluctuations depending on rainfall, with an annual mean flow of 3.5 $m^3\,s^{-1}$. During May–October 2014, the mean flow was 2.5 ± 1.8 $m^3\,s^{-1}$ (min. 0.5, max. 6.3 $m^3\,s^{-1}$), the mean water temperature was 12.5 ± 3.5 °C, and pH was 7–7.8. These values, and the water conductivity of 180–240 $\mu S\,cm^{-1}$, are typical for the Blanice River in this season. The release sites were in an area of broad-leaf trees and meadows at ~500 m asl. The sites had natural gravel banks and were similar with respect to substrate (gravel and stones), area (about 2000 m^2), and water velocity. The borders of sections consisted of natural shallow gravel riffles, making fish migration difficult and were ideal for installation of pulsed-DC electrofishing units as a barrier to escape from the sites during electrofishing sampling. Depth ranged from 10–80 cm and width from 7–11 m in all sites. The dominant fish species in the area are brown trout *Salmo trutta* m. *fario* L. and grayling. European bullhead *Cottus gobio* L., stone loach *Barbatula barbatula* L., roach *Rutilus rutilus* L., and gudgeon *Gobio gobio* L. were caught occasionally. The sites were under severe predation pressure from the great cormorant

Table 1. Number of grayling released at each site and tagging.

Release site	Hatchery	n	Colour of VIE tag	Position of VIE tag
I	Hu1 and Hy	100/100	Yellow/Red	Both sides of head
II	Hu1 and Hy	100/100	Yellow/Red	Left side of head
III	Hu1 and Hy	100/100	Yellow/Red	Right side of head
IV	Hu2 and Pa	100/100	Green/Orange	Right side of head
V	Hu2 and Pa	100/100	Green/Orange	Both sides of head
VI	Hu2 and Pa	100/100	Green/Orange	Left side of head

Hu = Husinec; Hy = Hynčice; Pa = Pardubice.

Phalacrocorax carbo throughout several winters beginning in 2008/9. Therefore, we supposed strong reduction of fish (especially grayling) populations in all sites.

2.2 Fish

Hatchery-reared grayling were randomly chosen from one-year-old fish reared on conventional dry food pellets from fingerlings in concrete tanks at the Husinec, Pardubice, and Hynčice hatcheries.

Fish from the Husinec Hatchery (Czech Anglers' Union) were progeny of resident wild broodstock from the Blanice River (hand stripped). The hatchery is supplied with water from the Blanice River and is 5–7 km from the release sites.

Hynčice and Pardubice fish were transported on 12 May 2014 to the Research Institute of Fish Culture and Hydrobiology in Vodnany. The Pardubice hatchery (Czech Anglers' Union), at ~220 m asl, is supplied with water from the Loučná River in East Bohemia (Labe basin) and is 250 km from the release sites (Fig. 1). The fish were progeny of resident wild broodstock of the Loučná River (hand stripped). The Loučná River is 81 km long with a drainage area of 724 km^2. The fish were transported in an aerated plastic tank, and duration of transport was approximately 3 h.

The Hynčice hatchery, at an elevation of ~420 m asl, is supplied with water from the Stěnava River in north-east Bohemia (Odra basin) and is 300 km from the release sites (Fig. 1). The Stěnava River is 62 km long with a drainage area of 594 km^2. Fish from this hatchery were progeny of wild broodfish originating from the Metuje River (Labe basin). The fish were transported in three plastic bags with oxygenated water, and duration of transport was about 4 h. Fish were held overnight (~15 h) at the Research Institute of Fish Culture and Hydrobiology in aerated 400 l aquaria in tap water until release.

2.3 Release

In their respective hatcheries prior to transport, fish were anaesthetized with 2-phenoxy-ethanol (0.2 ml l^{-1}), and Visible Implant Elastomer (VIE) tags (Northwest Marine Technology, Ltd., USA), using a different colour for each source group, were injected in small quantities under the transparent skin behind the eye, on the mandible, and the operculum. Fish were tagged on either the left or right side of the head or mid-mandible and behind both eyes to identify them according to release site. The tags were injected twice in each position as a precaution against loss. Fish from the Husinec hatchery were randomly divided and tagged as Husinec Groups 1 and 2 according to co-released fish group (Tab. 1). Fifty fish from each hatchery were randomly chosen, measured (standard length L_S, cm), and weighed (W, g) using a KERN Balance (type EMB 1200-1; max. 1200 g, d = 0.1 g) with a plastic bowl and measuring groove accurate to 1 mm. Fulton's condition factor ($K = W/L_S^{-3} \times 100$) was determined for each measured fish. After tagging, fish from the Husinec hatchery were placed in a flow-through system with water from the Blanice River in the hatchery area, and fish from Pardubice and Hynčice were placed in aquaria with tap water. The following morning (13 May), fish were transported to the release sites. Water from the Blanice River was used for the transport of fish from the Husinec groups in containers fitted with an oxygen injection system, and the duration of transport did not exceed 20 min. Tap water was used for transporting fish from the Hynčice and Pardubice groups in the same containers used for the Husinec groups, duration of transport was ~30 min. Before release, water from the river was slowly mixed with water in transport containers. Each site was stocked with 100 fish from the Husinec hatchery along with 100 from Hynčice (sites I–III) or Pardubice (sites IV–VI) (Tab. 1). Fish were released in groups in the central area of each site. The total number of stocked fish was 1200.

2.4 Recapture

Five months after release (20–21 October 2014), the sites and the stretches of river separating them, including approximately 1 km of the river downstream of the release area, were rigorously electrofished twice using two back-pack pulsed-DC electrofishing units (FEG 1500, EFKO-Germany). A further two backpack pulsed-DC electrofishing units (FEG 3000, EFKO-Germany) were situated at the upper border of each site to prevent fish from escaping upstream. All recaptured tagged fish were identified, measured, and weighed, and condition factor was determined. After measurement, fish from the Husinec groups were released near the point of capture, and fish from the Pardubice and Hynčice groups were released into a fishing area downstream of the surveyed area.

2.5 Statistical analyses

A one-way analysis of variance (ANOVA) was used to assess differences in weight, length, and K among fish groups prior to stocking and at recapture. Post hoc comparisons were

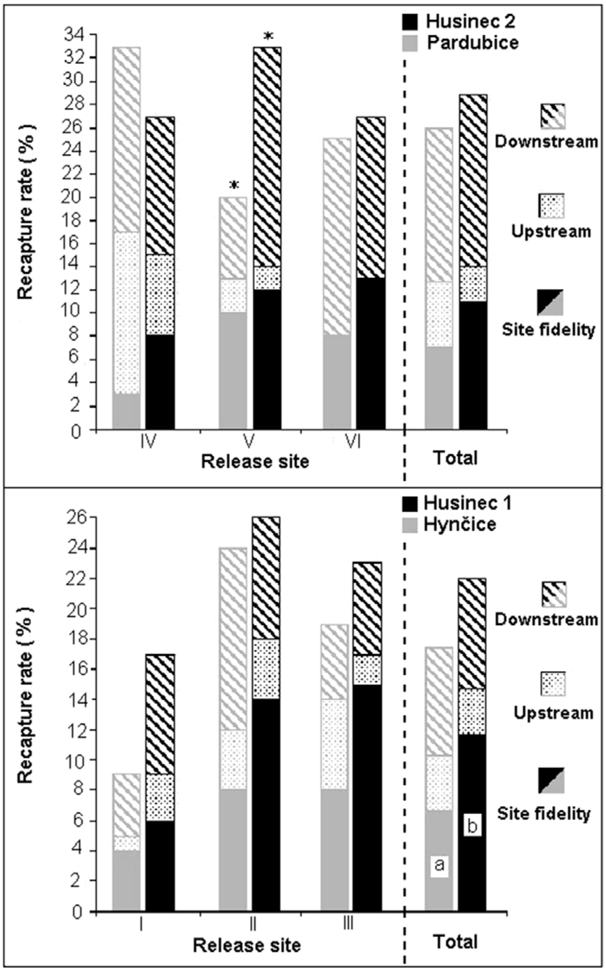

Fig. 2. Recapture rate (site fidelity = recapturing into release site/upstream = recapturing up of release site/downstream = recapturing down of release site) of stocked grayling in each site and total values (right column). *above bars indicates a significant difference ($P < 0.05$) in total recapture rate, different letters in bars indicate a significant difference ($P < 0.05$) in site fidelity/upstream/downstream recapture rate.

made by Tukey's honest significant difference test. Student's *t*-test was used to test for weight, length, and K differences among groups of grayling stocked in the same sites. Recapture rates and movement of tagged fish were compared with the Pearson and maximum likelihood χ^2 test. Significance was accepted for values of $p < 0.05$.

3 Results

Of the 1200 grayling tagged, 283 (23.6%) were recaptured. Wild grayling were occasionally captured, chiefly in the downstream areas (9 ind., SL 17–33 cm in Sites III–VI; 36 ind., SL 10–36 cm in Sites I and II). Higher density of brown trout in the lower river stretches was visually estimated during electrofishing. No significant differences in recapture rate were found between groups released within a given site (Sites I–III, Husinec 1, 22.0% v. Hynčice, 17.3%; Sites IV–VI, Husinec 2, 29.0% v. Pardubice, 26.0%), with the exception of

Table 2. Biometric data for tagged grayling before release: mean (±SD) standard length (L_S), weight (W), and Fulton's condition factor (K).

Group	Number of fish	L_S (cm)	W (g)	K
Pardubice	50	11.8 ± 1.4^a	19.6 ± 8.3^a	1.11 ± 0.12^b
Hynčice	50	10.6 ± 0.8^b	15.9 ± 3.9^b	1.30 ± 0.10^a
Husinec	50	10.6 ± 0.8^b	12.5 ± 2.6^c	1.05 ± 0.12^c

Within a column, different superscripts symbols indicate significant difference ($P < 0.05$) determined by post hoc Tukey test.

Table 3. Biometric data for tagged grayling after recapture: mean (±SD) standard length (L_S), weight (W), and Fulton's condition factor (K).

Group	Number of fish	L_S (cm)	W (g)	K
Pardubice	78	19.0 ± 1.2^b	90.1 ± 16.3^b	1.30 ± 0.11^b
Hynčice	52	18.3 ± 1.3^a	76.5 ± 15.2^a	1.23 ± 0.11^a
Husinec 1	66	18.4 ± 0.9^a	78.3 ± 12.9^a	1.25 ± 0.09^a
Husinec 2	87	18.9 ± 1.1^{ab}	90.8 ± 14.9^b	1.35 ± 0.13^c

Within a column, different superscripts symbols indicate significant difference ($P < 0.05$) determined by post hoc Tukey test.

Site V, which showed a significantly higher recapture rate ($\chi^2 = 4.34$; $df = 1$; $p = 0.037$) of local fish (Husinec 2, 33.0%) than distant strains (Pardubice, 20.0%).

Differences in recapture rate were found with respect to release site ($\chi^2 = 12.78$; $df = 3$; $p = 0.005$). Fish from the Hynčice group showed lower probability of being recaptured than fish released into upstream sites (IV–VI), Husinec 2 ($p < 0.001$) and Pardubice ($p = 0.010$). A significantly higher recapture rate of fish from Husinec 2 than Husinec 1 ($p = 0.049$) was also found.

Site fidelity, the recapture of marked fish in their original release site, was significantly higher ($\chi^2 = 4.504$; $df = 1$; $p = 0.034$) for Husinec 1 (11.7%) than for the Hynčice group (6.7%). Recapture downstream or upstream of release site did not differ among groups. Values of recapture rate are provided in Figure 2.

Significantly different weight ($F = 20.61$; $df = 2$; $p < 0.001$) and condition factor ($F = 71.15$; $df = 2$; $p < 0.001$) of fish before release were found among hatchery groups (Husinec groups combined). Initial SL of fish was higher ($F = 23.83$; $df = 2$; $p < 0.001$) in the Pardubice group than in Husinec and Hynčice groups (Tab. 2).

The weight and length of fish increased significantly in all groups over the course of the trial. Final weight and SL of groups released in the same site did not differ. Recaptured Husinec 2 and Pardubice fish were significantly heavier ($F = 17.29$; $df = 3$; $p < 0.001$) than other groups. Pardubice fish were also longer than fish from Husinec 1 and Hynčice at recapture (Tab. 3).

Significant differences ($F = 14.89$; $df = 3$; $p < 0.001$) were found in condition factor of recaptured fish. The Husinec 2 group exhibited higher K than the Pardubice group ($p = 0.042$). Final condition factor of fish from these two groups was significantly higher than fish from Husinec 1 ($p < 0.001$ vs. Husinec 2 and $p = 0.029$ vs. Pardubice) and Hynčice groups ($p < 0.001$ vs. Husinec 2 and $p = 0.005$ vs. Pardubice).

There were no significant differences in mean final weight, SL, and condition factor of fish groups released in the same site. Within each group, there were no differences in weight, SL, and condition among fish recaptured within, upstream, or downstream of their original release site.

4 Discussion

The study demonstrated no significant advantage of stocking one-year-old European grayling from a local hatchery in comparison with strains from distant localities as assessed five months post-release. The higher recapture rate of local stock released in one site was not noted in the other two sites stocked with these strains.

With regard to the crucial time for fish adaptation immediately after stocking and during the first weeks in the wild (Heggberget et al., 1993; Olla et al., 1994, 1998; Brown and Laland, 2001), the five month period between stocking and recapture in our study should have been sufficient to provide information of adaptation to the Blanice River environment, including natural prey intake. Our results are contrary to the higher recapture rates of brown trout stocked into streams near their rearing site than in distant areas reported by Johnsen and Hesthagen (1990), who attributed the difference to factors associated with transportation but suggested that the impact of local climatic conditions and/or water chemistry could not be discounted. In the present study, transportation from distant hatcheries was 3–4 h in oxygenated plastic bags. During marking and overnight before release, fish were held in aquaria with tap water and aerating pumps typical for transportation. The local strains of fish were held in flow-through tanks with water from the stocked river. A negative effect of transportation time and conditions was not observed. However, equalization of the initially significantly lower weight and condition factor of Husinec fish compared with the non-local fish released into the same sites may indicate an advantage for local fish. It also agrees with the findings of Jonsson et al. (1999), who found better growth of brown trout acclimated prior to stocking compared to fish released directly. The lack of difference in downstream migration rate is consistent with results of Thorfve (2002) who reported no significant influence of the acclimatization period on downstream migration of stocked grayling.

The recapture rates observed in all groups of fish in the present study were higher than recapture rate of hatchery-reared, and similar to recapture rates of pond-reared, yearling grayling in Turek et al. (2012). This may be attributed to reduction of territorial competition between wild and stocked grayling attributed to scarcity of wild fish due to cormorant predation in the previous winters. The lower recapture of fish released into downstream sites (I–III) may be explained by higher competition from wild fish. These sites were near a village, where predation by cormorants may have been reduced, as suggested by the higher numbers of wild grayling and brown trout caught in these sections during electrofishing. Similarly, Hanfland and Laggerbauer (2011) observed fair grayling population in urban areas of rivers otherwise affected by cormorants, although other environmental parameters were suboptimal. Given the similar morphology of all sections, the influence of abiotic factors on lower performance in groups stocked in downstream section is unlikely. Hart et al. (2014) pointed to the importance of familiarity on adaptation to changing environmental conditions in grayling juveniles: fish paired with familiar partners were significantly more likely to enter and forage in new, upstream habitats with potentially reduced risk of predation. Hence, our strategy of stocking several fish groups into a single site may have improved the post-release performance and should be recommended for grayling management.

Some studies have pointed to a negative impact of non-autochthonous stock on the genetic structure of local grayling populations (Duftner et al., 2005; Dawnay et al., 2011; Weiss et al., 2013), especially for watersheds with preserved genetic structure and diversity of wild populations. Persat et al. (2016) suggested that stocking of grayling has provided no reproductive benefits to wild populations, due to low long-term survival of stocked fish. In the Czech Republic, populations of European grayling in the Elbe basin show low genetic variability and were strongly affected by introgressive gene flow caused by previous fishery management and restocking (Havelka, 2009; Havelka et al., 2013). The populations used in the present study were genetically similar, belonging to the east-central European group (Gum et al., 2005) of grayling populations. The results suggest the feasibility of using fish from remote hatcheries within the Elbe drainage in Czech Republic for restoration of threatened grayling populations without additional deterioration of genetic variation. After re-establishment of a naturally reproducing population, further management practices should be focused on the development of conditions for natural spawning and local stock production, based on a combination of intensive autochthonous broodstock production and wild fish exploitation, as suggested by Randák (2014).

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