

Recapture and condition of pond-reared, and hatchery-reared 1+ European grayling stocked in addition to wild conspecifics in a small river

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ABSTRACT

Key-words:

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The relative performance of European grayling *Thymallus thymallus* reared in a hatchery on commercial dry feed or in a pond with natural food and their wild conspecifics, was assessed through recapture of tagged fish 5 months after their release into the Blanice River, Czech Republic. One-year old pond and hatchery reared fish from a resident broodstock were marked using Visible Implant Elastomer tags and released into 3 sections of river in May 2006. Wild one-year-old grayling were also tagged in these sections on the same days. The ratio of hatchery reared, pond reared, and wild fish was 1:1:1 in all sections. The recapture rate (hatchery 14.9%, pond 22.1%, and wild 51.3%) and site fidelity (hatchery 7.8%, pond 13.0%, and wild 35.1%) were significantly different among groups. Wild fish had a higher probability of recapture upstream of their original section than did hatchery or pond reared fish. Pond rearing was superior to conventional hatchery rearing for subsequent stocking of 1+ grayling in running water. Initially different mean condition factors were similar in all groups at recapture, suggesting adaptation of the artificially reared fish that remained in the river sections surveyed.

RÉSUMÉ

Recapture et condition d'ombres communs 1+ élevés en étang ou en pisciculture déversés en complément de congénères sauvages dans une petite rivière

Mots-clés :

Thymallus thymallus,
gestion de
rivière,
alevinage,
recapture,
ombre

La performance relative des ombres communs *Thymallus thymallus* élevés dans une écloserie avec des aliments secs commerciaux ou dans un étang avec des aliments naturels et de leurs congénères sauvages a été évaluée par la recapture de poissons marqués 5 mois après leur déversement dans la rivière Blanice, République tchèque. Des poissons d'un an issus de géniteurs sauvages, élevés en étang ou en écloserie, ont été marqués au moyen d'implants visibles élastomère et déversés en 3 sections de la rivière en mai 2006. Des ombres sauvages d'un an ont également été marqués dans ces sections ces mêmes jours. Le ratio de poissons d'élevage de pisciculture, d'étangs, et sauvages était dans toutes les sections 1:1:1. Le taux de recapture (écloserie de 14,9 %, 22,1 % étang, et sauvage 51,3 %) et la fidélité au site (écloserie de 7,8 %, d'étang de 13,0 %, et sauvage de

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35,1 %) étaient significativement différents entre les groupes. Les poissons sauvages avaient une plus forte probabilité de recapture en amont de leur section d'origine que les poissons d'écloserie ou d'étang. L'élevage en étang a été plus performant que l'élevage en écloserie classique pour le déversement d'ombres 1+ en rivière. Les différents facteurs de condition étaient les mêmes dans tous les groupes à la recapture, ce qui suggère une bonne adaptation des poissons élevés artificiellement qui restaient dans les sections des rivières étudiées.

INTRODUCTION

European grayling (*Thymallus thymallus* L.) is native to the Czech Republic (Balon, 1962; Lusk, 1975), but stocks in streams have been declining for many years. To re-establish threatened or extinct populations and expand the recreational fishery, hatchery-reared fish are commonly stocked. The success of stocking fish in natural waters depends on a variety of factors (Cowx, 1994). Hatchery-reared salmonids often have slower growth and lower fecundity and survival in the wild than do resident wild fish (Weber and Fausch, 2003). This may be partly due to stress effects of transportation (Jonsson *et al.*, 1999; Finstad *et al.*, 2003) and competition with wild residents, in which stocked fish generally lose conflicts – the so-called 'prior residence effect' (Rhodes and Quinn, 1998; Johnsson *et al.*, 1999). Salonen and Peuhkuri (2004) observed lower levels of aggressive behaviour in hatchery strains of *T. thymallus* than in the wild strains at the age of 0+ years, with the differences remaining to the age of 1+, when the strains had been reared for a year under common hatchery conditions.

Rearing methods of fish stock is the critical factor in post-stocking behaviour and survival. Studies comparing wild, hatchery-reared, and pond-reared fish have shown that stocked fish have lower survival rates than wild fish (e.g. Miller, 1954; Vincent, 1960; Flick and Webster, 1964; Ersbak and Haase, 1983; Weiss and Schmutz, 1999). Huet, (1986) reported that fish reared in ponds were more suitable for stocking into natural waters, because pond-reared fish have learned to feed on natural prey. Carlstein (1997) found that *T. thymallus* reared in a natural pond showed higher post-stocking survival in a lake than fish reared in a conventional hatchery. Näslund (1992) observed higher post-stocking survival in pond-reared brown trout compared to hatchery-reared. Johnsen and Ugedal (1986), however, found no long-term differences in post-stocking feeding of hatchery-reared and wild brown trout. Similarly, no differences were found between pond- and hatchery-reared brown trout in post-stocking feeding or in recapture rate (Johnsen and Hesthagen, 1990; Johnsen and Ugedal, 1990). Turek *et al.* (2010a) reported a lower recapture rate of hatchery- and pond-reared 2+ *T. thymallus* compared to their wild conspecifics in a small stream, with no differences in growth between the groups six month post-stocking. Some further evidence from Magee and Byorth (1994) and Kaya and Jeanes (1995) showed a high degree of post-stocking downstream dispersal in juvenile Arctic grayling (*Thymallus arcticus* Pallas), similar to results reported for *T. thymallus* (Carlstein and Eriksson, 1996; Thorfve and Carlstein, 1998).

To improve the design of future restocking programmes, increased knowledge about post-stocking performance of fish of varying ages and origins is needed. In the present study, pond and hatchery reared 1+ *T. thymallus* were released into three sections of the Blanice River in South Bohemia (Czech Republic). The aim of this study was to investigate the impact of the rearing technique on post-stocking growth and survival in this age category of *T. thymallus* and to compare performance of stocked fish with the resident conspecifics in the experimental sections.

MATERIALS AND METHODS

> STUDY AREA

The Blanice River in South Bohemia is 93 km long, with a drainage area of 860 km². Three experimental sections of 150–190 m were located in a 5 km section of the river (river km 53–58)

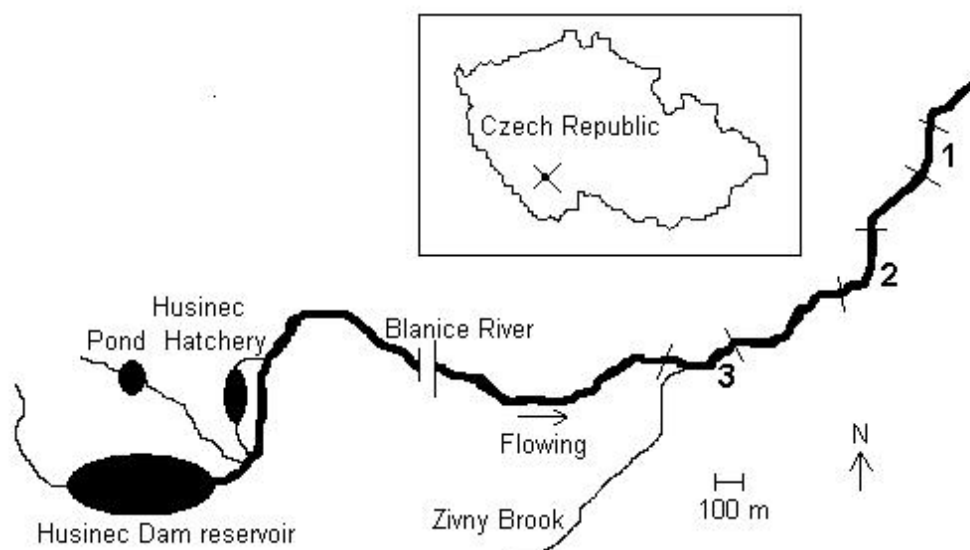


Figure 1

Schematic map showing the locations of experimental sections (1–3), hatchery and rearing pond.

in a protected area (fishing prohibited) downstream of the Husinec Dam reservoir (37 ha; $2.5 \times 10^6 \text{ m}^3$) (Figure 1). In this part of the river, water flow is characterized by wide seasonal fluctuations, with an annual mean flow of $3.5 \text{ m}^3 \cdot \text{s}^{-1}$. During the experimental period (May–October 2006), the mean (\pm SD) flow was $2.5 \pm 2.8 \text{ m}^3 \cdot \text{s}^{-1}$, the mean (\pm SD) water temperature was $12.5 \pm 3.5 \text{ }^\circ\text{C}$, and pH was 7–7.8. These values, and the water conductivity between $180\text{--}240 \mu\text{S} \cdot \text{cm}^{-1}$, are typical for the Blanice River in this season. The experimental sections are located in the countryside with broad-leaf trees and meadows at an elevation of about 500 m above sea level. The sections had natural gravel banks and were similar with respect to bottom substrate (gravel and stones), area ($1200\text{--}1500 \text{ m}^2$), and water velocity. Conditions were optimal for installation of pulsed-DC electrofishing units as barriers against escape of fish from the sections during electrofishing sampling. Depth range was 10–80 cm in all sections. The dominant fish species are brown trout (*Salmo trutta m. fario* L.) and grayling (*Thymallus thymallus* L.). Common sculpin (*Cottus gobio*), stone loach (*Barbatula barbatula* L.), roach (*Rutilus rutilus* L.), and gudgeon (*Gobio gobio* L.) are caught occasionally.

EXPERIMENTAL FISH

All experimental fish were first generation progeny of wild broodstock resident in the Blanice River. One year-old *T. thymallus* originating from artificial spawning were reared from fingerling stage in ponds (pond fish) or in concrete tanks (hatchery fish). Pond fish were held in a natural pond of c. 1 ha supplied with water from a tributary of the Blanice River. Feeding was from naturally-occurring sources (zooplankton, benthos, terrestrial invertebrates). Hatchery fish were held in concrete tanks in the Husinec Hatchery (Czech Anglers' Union) supplied with water from the Blanice River and were fed on commercial dry food pellets. Both rearing facilities were situated c. 5–7 km upstream of the study area.

Table I

Numbers of fish and their tagging in each experimental section at the beginning of experiment.

Section	Group	Number of fish stocked/measured	Colour of VIE tag	Position of VIE tag
I	Wild	44/44	Green	Left side of head
	Pond	44/15	Red	(operculum and behind eye)
	Hatchery	44/15	Pink	and left side of mandible
II	Wild	58/58	Green	Right side of head
	Pond	58/15	Red	(operculum and behind eye)
	Hatchery	58/15	Pink	and right side of mandible
III	Wild	52/52	Green	Both side of head
	Pond	52/15	Red	(operculum and behind eyes)
	Hatchery	52/15	Pink	and middle of mandible

> SAMPLING

Wild resident *T. thymallus* were captured by electrofishing. Sections were rigorously sampled twice on 15 May, 2006 using two back-pack pulsed-DC electrofishing units (FEG 1500, EFKO-Germany). A further two pulsed-DC electrofishing units (FEG 3000, EFKO-Germany) were situated at the upper border of the section to prevent fish from escaping upstream. Captured wild *T. thymallus* of the target age group were anaesthetized with 2-phenoxy-ethanol (0.2 mL·L⁻¹), measured (standard length L_S , cm), weighed (weight W , g), using the KERN Balance (type EMB 1200-1; max. 1200 g, $d = 0.1$ g) with plastic bowl and the measuring groove (accurate to 1 mm), and tagged [visible implant elastomer (VIE), Northwest Marine Technology, Ltd., USA]. Fish were released near the point of capture after they fully recovered equilibrium and showed spontaneous swimming activity (c. 5 min after tagging). Population density of wild target fish was similar (approximately 0.05 individuals m⁻²) in all sections. Artificially reared *T. thymallus* were stocked (in 2–3 small groups) in the central part of sections on 16 May 2006. Water from the Blanice River was used for the transport in containers fitted with an oxygen injection system, and the duration of transport did not exceed 20 min. The fish were tagged as with wild graylings before release. The VIE tags (different colours for hatchery-reared, pond-reared, and wild) were injected in small amounts underneath the transparent skin behind the eye, on the mandible, and the operculum. Fish were tagged on either the left or right side of their head or mid-mandible and behind both eyes to identify them according to section (Table I). During marking, all fish were anaesthetized with 2-Phenoxyethanol (0.2 mL·L⁻¹). Forty-five fish from each cultured group (15 randomly chosen fish for each section) were measured (L_S , cm) and weighed (W , g).

The number of stocked fish from each group (hatchery and pond) was the same as the number of tagged wild *T. thymallus* of similar age in a given section. Consequently, the population of *T. thymallus* of this age category tripled in each experimental section. The total number of stocked fish was 154 individuals from each rearing method.

Five months after release (19–20 October 2006), the post-stocking performance of fish was evaluated. The fish were recaptured by the same method and with the same equipment described above. The experimental sections along with the sections between them, including approximately 2 km of the river upstream and downstream of the experimental area, were electrofished thoroughly. All tagged fish were identified, measured, and weighed and released near the point of capture. Initial and final Fulton's condition factor ($K = WL_S^{-3} \times 100$) was determined for each recaptured fish. Site fidelity was defined as recapture of fish in the sections where they were originally released. Otherwise, the direction of their post-stocking dispersal (upstream or downstream) was recorded.

> STATISTICAL ANALYSES

All data were analysed with the aid of the statistical package SAS (version 9.1; SAS Institute Inc.). We applied an analysis of categorical repeat measures based on the generalized

estimating equation (GEE) approach (Liang and Zeger, 1986) using the GENMOD procedure (SAS, version 9.1) with binomial distributions. In this study, the GENMOD procedure was designed to estimate the probability that fish (i) would be recaptured; (ii) would display site fidelity; (iii) would disperse downstream from the release section; and (iv) would disperse upstream from the release section. The explanatory variable 'origin of fish' was categorical, containing three classes (pond-reared fish, hatchery-reared fish, and wild fish).

Separate linear mixed models (LMM) were applied for the following dependent variables: weight (LMM I), standard length (LMM II), and condition factor K (LMM III and IV). LMM III was designed to compare the effect of fish origin before stocking and at recapture. LMM IV was designed to estimate the effect of fish origin combined with site fidelity at the end of the experiment. To randomize the effect of the river section, all analyses were performed using mixed model analysis, with river section as random factor, using PROC MIXED. The fixed effects were 'origin of fish' classes, 'time interval' (start, end), and 'site fidelity' (yes or no). The significance of each effect (including interactions) in the LMM was assessed by the F -test, with sequential dropping of the least significant effect, starting with a full model. The significance of each fixed effect in the mixed GLMM models was assessed by the F -test. Least-squares-means (LSM; also referred to as 'adjusted means') were computed for each class, and differences between classes were tested by t -test. For multiple comparisons we used the Tukey-Kramer adjustment. The degrees of freedom were calculated using the Kenward-Roger method (Kenward and Roger, 1997).

RESULTS

Of the 462 grayling tagged, 136 (29.4%) were recaptured five month after stocking. Wild fish showed a higher probability of being recaptured ($X^2 = 106.43$; $df. = 2$; $P < 0.001$; Figure 2a) and displaying site fidelity ($X^2 = 79.20$; $df. = 2$; $P < 0.001$; Figure 2b) than either of the two cultured fish groups. The probability of being captured and displaying site fidelity was higher in pond- than in hatchery-reared fish ($P = 0.0232$; $P = 0.0364$, respectively). Wild fish also showed higher probability of being recaptured upstream of their original section than did reared fish ($X^2 = 33.99$; $df. = 2$; $P < 0.001$; Figure 2c). The difference between groups of cultured fish in upstream recapture was not significant. Fish origin did not influence downstream dispersal of tagged graylings.

The weight and length of fish increased significantly in all groups over the course of the experiment. Wild fish were heavier ($F_{5,378} = 530.29$, $P < 0.001$) and longer ($F_{5,378} = 766.69$, $P < 0.001$) than reared fish at the beginning as well as at the end of the experiment (Tables II and III). Lower L_S of hatchery-reared fish in comparison with the pond-reared were recorded at the beginning of experiment only.

Wild fish showed higher condition factor (K) than cultured at the beginning of the experiment ($F_{5,378} = 51.68$, $P < 0.001$; Tables II and III), with significant differences between pond- and hatchery-reared. All groups of fish showed equal condition at the end of the experiment. This was the result of increased condition of reared fish along with decreased condition of wild fish. The wild and hatchery fish recaptured outside of their original section, regardless of direction, showed lower K than fish exhibiting site fidelity ($F_{5,136} = 4.04$, $P = 0.002$; Figure 3).

DISCUSSION

Lower recapture of pond and hatchery-reared *T. thymallus* released into a natural stream compared to their wild conspecifics corresponds with published reports of many researchers (e.g. Ersbak and Haase, 1983; Arias *et al.*, 1995; Weiss and Schmutz, 1999) who also found lower post-stocking survival of artificially-reared fish. Competitive superiority of resident salmonids over introduced fish (Brännäs, 1995; Glova and Field-Dodgson, 1995), known as the 'prior residence effect' (Huntingford and DeLeaniz, 1997; Rhodes and Quinn, 1998), was a possible reason for lower recapture rate of artificially reared fish in comparison with the wild in the

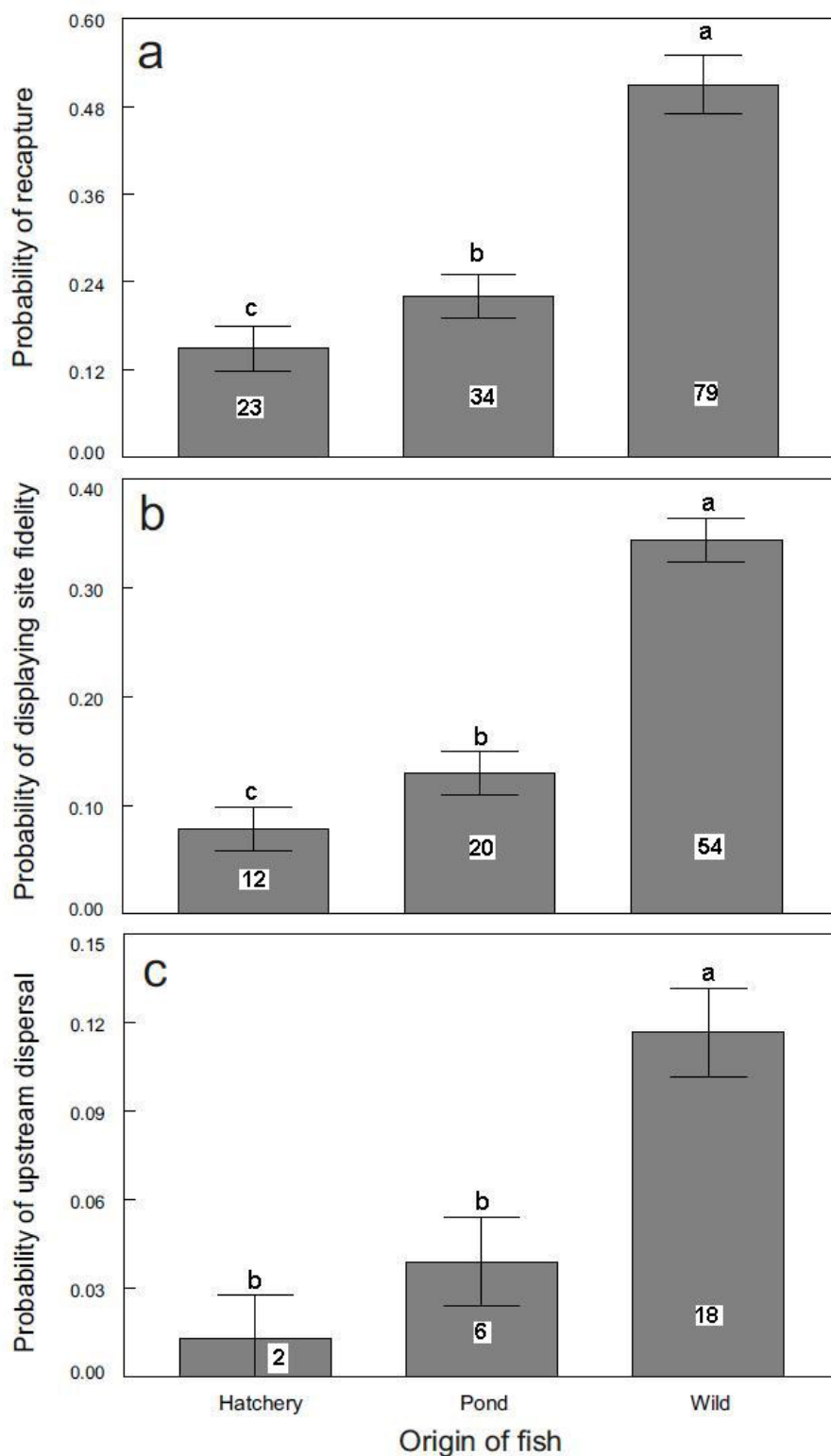


Figure 2

Predicted values (with confidence intervals) resulting from logistic regression model testing (a) probability of recapture, (b) displaying site fidelity and (c) upstream dispersal in relation to the origin of fish. Number of fish given is inside each column of the graph. Different letter above columns indicate a significant difference ($P < 0.05$).

Table II

Post hoc Tukey results displaying mean (\pm SD) standard length (L_S), weight (W), Fulton's condition factor (K) of the tagged grayling.

Group	Number of fish	L_S (cm)	W (g)	* K
Wild	154	15.0 \pm 1.2 ^a	46.5 \pm 11.9 ^a	1.37 \pm 0.23 ^a
Pond	45	12.1 \pm 1.0 ^b	18.4 \pm 5.6 ^b	1.02 \pm 0.21 ^b
Hatchery	45	10.9 \pm 0.9 ^c	11.4 \pm 5.3 ^b	0.85 \pm 0.23 ^c

Within a column, different superscripts letters indicate a significant difference ($P < 0.05$).

* $K = (WL_S^{-3}) \times 100$.

Table III

Post hoc Tukey results displaying mean (\pm SD) standard length (L_S), weight (W), Fulton's condition factor (K) of the recaptured grayling.

Group	Number of fish	L_S (cm)	W (g)	* K
Wild	79	21.3 \pm 1.4 ^a	123.0 \pm 24.2 ^a	1.27 \pm 0.17 ^a
Pond	34	19.2 \pm 1.0 ^b	89.3 \pm 15.7 ^b	1.26 \pm 0.15 ^a
Hatchery	23	19.3 \pm 1.1 ^b	88.0 \pm 17.6 ^b	1.22 \pm 0.20 ^a

Within a column, different superscripts letters indicate a significant difference ($P < 0.05$).

* $K = (WL_S^{-3}) \times 100$.

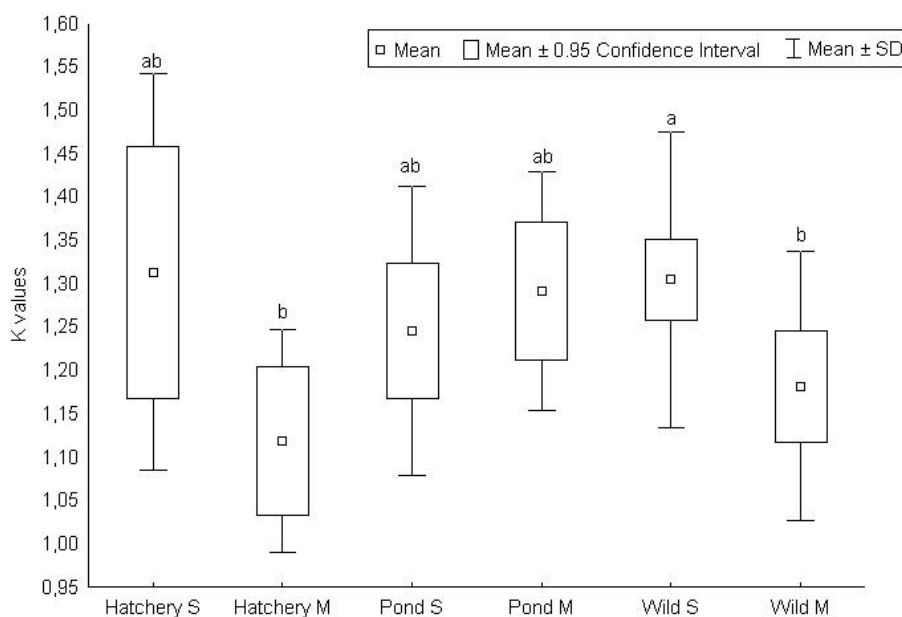


Figure 3

Relationship between condition factor (K) and origin of fish recaptured in (sedentary-S) and out (migratory-M) of their original section at the end of experiment. Different letter above columns indicate a significant difference ($P < 0.05$).

present study. The higher initial weight was an advantage for wild fish. The effect of individual weight on territorial competition has been demonstrated earlier in brown trout fry (Johnsson *et al.*, 1999). In the present study, the differences in initial weight among wild and reared fish can be explained by lack of natural prey in hatchery fish and/or higher rearing density in artificially reared fish. The influence of rearing method (pond vs. hatchery) on recapture rates of artificially reared fish in this experiment is consistent with some published results (Huet, 1986; Näslund, 1992; Carlstein, 1997). In contrast, Johnsen and Hesthagen (1990) reported that the rearing method is not critical to the recapture rate of stocked brown trout.

Turek *et al.* (2010a) also found no effect of rearing method on the recapture rate of two-year-old grayling in the Blanice River. However, in that study the population density of the

monitored category was doubled. It is possible that the tripled stocking density in the present study led to the differences in recapture rate between artificially reared groups of grayling. Also the age, 1+ in the present study vs. 2+ in Turek *et al.* (2010a), could be of importance, suggesting that the 'training' in a pond is more important for younger fish. Higher losses in hatchery-reared fish may also be associated with predation, as otters and herons occur in the study area. Wild and pond fish had been exposed to predators before stocking whereas hatchery fish had no prior experience. Several studies have demonstrated the importance of experience on the development of adequate anti-predator behaviour (e.g. Steward and Bjorn, 1990; Olla *et al.*, 1998). Furthermore, unintentional selection could reduce adaptability of hatchery-reared fish on natural conditions in the present study. The hatchery environment often favours different traits than natural selection in the wild. Therefore, good performance during hatchery rearing may indicate reduced performance in food-limited natural conditions (Saikkonen *et al.*, 2011).

The differences seen in initial mean body weight between wild and both groups of reared fish remained significant until the end of the experiment; however relative weight increase was comparable among all groups. This indicates that hatchery-reared fish were able to learn to feed on natural prey. This could be the result of social learning. Brown and Laland (2001) demonstrated that artificially cultured fish can learn to feed on novel prey by observing a trained conspecific 'demonstrator'. Observed results pointed to competition between stocked and wild fish for territory rather than for food. Sufficient prey for the increased density of fish in the Blanice River after stocking was indicated in other experiments (Turek *et al.*, 2010a, 2010b). However, this cannot be generalized; observations published by Weiss and Schmutz (1999) indicated that abiotic factors in heterogeneous biotopes substantially affected growth of stocked fish. The possible short-term weight loss reported by Thorfve (2002) in stocked grayling was not observed during the 5 months of the present experiment.

Although the condition factor of reared grayling was initially lower, it was equal to that of wild fish at recapture, suggesting that those artificially reared fish that remained in the experimental sections adapted to local conditions. However, adverse effects of the stocking on wild fish were also demonstrated, as their condition factor was lower at the end of the experiment. Observed influence of migration on *K* values can be explained through higher energy consumption for territorial competition between migratory and resident fish in a new area (Huntingford and De Leaniz, 1997; Rhodes and Quinn, 1998). This strengthens the possibility that habitat competition is one of the primary limiting factors for survival of stocked fish in the Blanice River.

Results of the present study suggest that stocking of artificially reared one-year-old grayling can be used to boost weakening populations of this species. Rearing in a pond was shown to be superior to conventional hatchery rearing for subsequent stocking of 1+ graylings in running water. However, stocked fish displayed lower recapture and site fidelity compared to wild resident fish. Also an adverse effect of stocking on wild fish was demonstrated. Therefore facilitation of natural spawning should be preferentially practised and supported.

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