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Factors influencing movement and behaviour of animals in riverine environment

Faktory ovlivňující pohyb a chování živočichů v říčním prostředí

PH.D. THESIS

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I declare that this thesis has not been submitted for the purpose of obtaining the same or any other academic degree earlier or at another institution. My involvement in the research presented in this thesis is expressed through the authorship order of the included publications and manuscripts. All literature sources I used when writing this thesis have been properly cited.

Petra Horká Prague, September 2013

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Publications and manuscripts

Part I

Chapter I

Kulíšková, P., Horký, P., Slavík, O., Jones, J. I. (2009). Factors influencing movement behaviour and home range size in ide *Leuciscus idus*. *Journal of Fish Biology* **74**, 1269–1279.

Chapter II

Horká, P., Horký, P., Randák, T., Turek, J., Slavík, O. Unlimited response to environmental factors: a telemetry study on the behaviour of wild and hatchery-reared European grayling *Thymallus thymallus*. *Unpublished manuscript,* 15 pp.

Chapter III

Horká, P., Horký, P., Slavík, O., Opatřilová, L. (2012). Diurnal behavioural patterns and spread of the Ponto-Caspian invader *Hemimysis anomala* G. O. SARS, 1907 (Crustacea, Mysidacea) in the Elbe River, Czech Republic. *International Review of Hydrobiology* **97**, 454–462.

Part II

Chapter IV

Horký, P., **Horká, P.**, Jurajda, P., Slavík, O. (2013). Young-of-the-year (YOY) assemblage sampling as a tool for assessing the ecological quality of running waters. *Journal of Applied Ichthyology* **29**, 1040–1049.

Chapter V

Musil, J., Horký, P., Slavík, O., Zbořil, A., **Horká, P.** (2012). The response of the young of the year fish to river obstacles: Functional and numerical linkages between dams, weirs, fish habitat guilds and biotic integrity across large spatial scale. *Ecological Indicators* **23**, 634–640.

Appendix

Horká, P., Ibbotson, A., Jones, J. I., Cove, R. J., Scott, L. J. (2010). Validation of scale-age determination in European grayling *Thymallus thymallus* using tag-recapture analysis. *Journal of Fish Biology* **77**, 153–161.

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Abstrakt (in Czech)

Tekoucí vody představují velmi variabilní prostředí a organismy, které je obývají, využívají různé adaptační mechanismy, které umožňují jejich existenci a rozmnožování. Chování a migrace živočichů patří mezi klíčové mechanismy umožňující přizpůsobení k podmínkám prostředí a jejich změnám. Ve své disertační práci jsem se zabývala zejména abiotickými a antropogenními faktory, které ovlivňují časovou a prostorovou distribuci a chování živočichů v říčním prostředí. Disertační práce se skládá ze dvou částí: první část je složena ze tří kapitol, které zkoumají které abiotické faktory a jakým způsobem ovlivňují chování živočichů. První dvě kapitoly presentují výsledky rozsáhlých telemetrických studií, popisujících diurnální chování a migrace jelce jesena (*Leuciscus idus*) a lipana podhorního (*Thymallus thymallus*). První kapitola představuje výsledky ročního sledování diurnální a sezónní variability v chování jelce jesena v řece Labi, druhá kapitola je výsledkem tříletého sledování juvenilních lipanů v průběhu podzimního a zimního období. V této studii jsme se dále zaměřili na rozdílnosti v chování divokých lipanů a lipanů ze sádek. Třetí kapitola zkoumá diurnální chování a šíření invazního korýše, pontokaspického vidlonožce *Hemimysis anomala*. Druhá část disertační práce zahrnuje dvě publikace založené na hodnocení společenstev juvenilních ryb: první je zaměřena na hodnocení ekologického stavu tekoucích vod pomocí metody vyhodnocující složení společenstev juvenilních ryb pro potřeby Rámcové směrnice o vodách. Druhá studie vyhodnocuje vliv příčných překážek na složení rybích společenstev v tocích s různou velikostí a ekologickou kvalitou. Cílem poslední studie, jež je zařazena jako příloha disertační práce, je ověření správnosti široce rozšířené metody určování věku lipana podhorního pomocí šupin. Ačkoli je znalost věkové struktury podstatná pro správný odhad složení populací a přiměřené hospodaření na tocích, moje výsledky prokazují, že u konkrétních druhů mohou být odhady věku na základě šupin značně podhodnocené.

Abstract

Animals living in riverine environments are influenced by various abiotic and biotic factors which shape their behaviour. Among the most distinctive behavioural traits by which animals react and adapt to environmental variability is movement behaviour. In my study I focused on movement and distribution of several species and their response to environmental and anthropogenic factors. The thesis consists of two parts: the first part synthesizes studies on behaviour of European grayling *Thymallus thymallus*, ide *Leuciscus idus* and an invasive crustacean *Hemimysis anomala*; the objectives of individual projects were to determine which environmental factors influence the species' behaviour and how. The first two chapters present extensive radio-telemetry studies which examined diel movements and migration of ide (one year observation in the River Elbe) and European grayling (study conducted in autumn-winter periods of three consecutive years in the River Blanice, focusing on differences between juveniles of wild and hatchery origin). The third study was also conducted in the Elbe and evaluated the diurnal behavioural patterns and spread of the Pontocaspian mysid shrimp *Hemimysis anomala*. The second part of the thesis consists of two chapters focusing on young-of-the-year (YOY) fish assemblages: the first deals with YOY assemblage sampling as a tool for assessing the ecological quality of running waters for the requirements of the Water Framework Directive, the second assesses the response of fish community to river obstacles and evaluates the composition of YOY fish from rivers with different ecological status. The last study, included in my dissertation as an appendix, focus again on European grayling. Its aim was the validation of a widely used scale-age determination method substantial for the understanding the dynamics of fish population and appropriate management of fish stocks. My results show, however, that the scale-based age estimated may be substantially biased for particular species.

1 General Introduction

Animals living in running waters are confronted by a wide range of environmental challenges which demand great physiological and behavioural flexibility of individuals. Among the most obvious traits enabling adaptation to various environmental conditions belongs the movement behaviour. For many species utilising freshwater habitats, movement is of substantial importance for their reproduction and survival, as they migrate between habitats essential for reproduction, feeding, or for predator avoidance. Such movements may range from local movements such as daily foraging (Snedden *et al.*, 1999; Koed *et al.*, 2000) and diurnal vertical migration (Lampert, 1989), to shifts between habitats with age or season (Lucas & Batley, 1996; Sempeski *et al.*, 1997), or long distance migrations such as spawning migration in fish (Molls, 1999; Geeraerts *et al.*, 2007). During the lifecycle, the requirements of individuals may change, necessitating a change in location, and individuals can maximize their fitness if they appropriately move between most suitable habitats (Northcote, 1978; Molls, 1999).

Movement behaviour of animals may be assessed both on spatial and temporal scales. The distribution of individuals within a population reflects their response to various factors, both environmental and biological. Among key environmental factors determining animal movements in riverine environment belong the day length, season (Geeraerts *et al.*, 2007) and the characteristics of habitats such as flow (Schlosser, 1985), water temperature (Lucas & Batley, 1996; Geeraerts *et al.*, 2007) or the physical structure of the locality (Slavík *et al.*, 2005). The crucial biotic factors are predator-prey interactions, prey availability, inter- and intraspecific competition, and the presence of other conspecifics such as suitable mates (Wootton, 1998). The movement behaviour of many aquatic invertebrates and fish is also influenced by anthropogenic factors, such as water pollution, suspended sediment loads associated with eutrophication, or artificial barriers.

Although the movements and migrations of animals living in running waters have long been the subject of considerable interest, there is not yet a clear understanding of all factors under which various movement patterns are demonstrated. For example, little is still known about diel movement behaviour of many fish species and freshwater invertebrates, and about environmental cues responsible for behavioural variability expressed within diel and/or seasonal cycle. Such knowledge is simultaneously crucial to reveal the potential impact of anthropogenically induced changes in riverine environment.

The studies included in my thesis examine mostly temporal and spatial patterns of movement or distribution of animals living in riverine environment and their response to environmental variability. The main topics covered are (1) behaviour and movement of animals within diel and seasonal cycle, (2) environmental factors influencing demonstrated behavioural patterns, and (3) the impact of selected factors, particularly turbidity, human-constructed obstacles, and rearing environment, on behaviour. These are dealt within five studies, which examine these issues from several points of view. Furthermore, I include as an appendix an additional study that originated from my involvement in research of ecology of European grayling *Thymallus thymallus*, and focuses on validation of commonly used method for its age determination.

Home range

Many fish species exhibit well developed home range behaviour. Evidence of fish home ranges comes from repeated observations of tagged individuals in the same area and from radio tracking studies which were further reinforced by observations of homing to the area of original capture in displacement and release studies (Lucas & Baras, 2001).

Home range may be characterised as an area to which animals usually confine their daily activities. There are many concepts on home range, however the basic and the most commonly cited definition of an animal's home range is that of Burt (1943) that "home range is an area traversed by the individual in its normal activities of food gathering, mating and caring for young". Home range has also been characterised as an area that meets the energy requirements of the individual (Jewell, 1966). For freshwater fishes, home ranges have been demonstrated in a wide range of species (e.g. Rodríguez, 2002; Penczak, 2006; Slavík *et al.*, 2007). A fish adjusts its home range to include habitats which fulfil their requirements; most frequently these include areas used for feeding, reproduction, and as a refuge (Lucas & Baras, 2001).

Historically, many freshwater-resident fish in rivers were regarded as non-migratory, displaying strong site fidelity and occupying restricted home ranges (Gerking, 1959; Rodríguez, 2002). Such assumption has become known as the *restricted-movement paradigm* (sensu Gowan *et al.*, 1994). However, such presumptions were mainly based on mark-recapture studies, which provided only very course information about the nature of movements and causes of migration. Later, it was found that the home ranges of fish are not always spatially constant. An individual may utilize one home range and then relocate/display home range shift and utilize a new home range, or undertake large-scale movements and subsequently return to the original home site, displaying homing behaviour (Baade & Fredrich 1998; Lucas & Baras, 2001). For example, flathead catfish *Pylodictis olivaris* exhibit a high degree of site fidelity to large woody debris and riprap habitats from spring to fall, indicating that these habitat types provide important cover for the majority of the year, while main-channel pool habitats with coarse substrates provide refuge with greater water depth and reduced river flow rates during winter months when fish are inactive (Daugherty & Sutton, 2005).

Home range shift might occur in various contexts, including disturbances (e.g. floods, drought, capture and release by humans), behavioural interactions (e.g. arrival of new predators or competitors), decrease in habitat profitability, or when a new suitable area is located. Home range shift might also occur after specialized movements associated with the species' life history, such as spawning migrations or relocation to overwintering or refuge habitats (Snedden *et al.*, 1999; Horký *et al.*, 2007*b*). It is suggested that such movements provide great adaptive advantage for survival and mechanism improving lifetime fitness (Lucas & Baras, 2001).

The *home-range size* depends on several factors which vary with fish species. Considerable attention has been given to the factors determining the areas of home ranges defended by aggressive behaviour (territories). Important variables include resource availability, competitor density, and body size of the territory holder (Harestad & Bunnel, 1979; Hixon, 1987; Grant *et al.*, 1998). Home range sizes may also reflect habitat productivity (Hansen & Closs, 2005), season (Slavík *et al.*, 2007) or the distribution of refuges and habitat type (Slavík *et al.*, 2005). For example, home range size of Prussian carp *Carassius auratus gibelio*, was largest in floodplains, smaller in the main river channel, and minimal in a navigation canal (Slavík & Bartoš, 2004).

Diel movements/activity

The ability of fish to forage, detect predators or attract mates may vary with the daily cycle of light and dark. Species are usually active during limited periods within the diel cycle, being diurnal, nocturnal, or crepuscular (Wootton, 1998). The preferred period often differs among related species within the same habitat. For example, peaks of diel activity of chub *Leuciscus cephalus*, bream *Abramis brama*, and white bream *Blicca bjoerkna* occurred during daylight, whereas Prussian carp *Carassius auratus gibelio*, was active mostly at night (Horký *et al.*, 2007*a*). Activity rhythms may change during the course of the year, with alternating diurnal/nocturnal peak of activity. During summer, peaks in activity of barbel *Barbus barbus,* tended to occur in early morning and late evening, although there was substantial variability between sexes and months. In winter, mean daily activity was less than 20% of peak summer levels and fish diel activity exhibit a single peak towards dusk (Lucas & Batley, 1996). In the study of European catfish *Silurus glanis* in the Berounka River, nocturnal activity occurred in adult catfish in autumn, whilst during winter and spring, the maximum movement was recorded during the day (Slavík *et al.*, 2007). Likewise, autumn-winter period is often reported to be associated with night time activity in salmonids (Fraser *et al.*, 1993; Valdimarsson & Metcalfe, 1998). The restricted activity in winter is apparently an efficient strategy to cope with seasonally unfavourable environmental conditions, when overwintering fish have to face low water temperature and/or low food concentration (Ultsch, 1989; Bauer & Schlott, 2004).

Peaks of activity may also reflect variations in behavioural strategies of species. In a large lowland Australian river murray cod *Maccullochella peelii peelii,* appear largely sedentary except during spawning period in comparison to common carp *Cyprinus carpio* (Jones & Stuart, 2007). The authors suggested that interspecific differences in movement may be related to different feeding behaviours, as cod are lie-in-wait predators, using cover to ambush prey (including carp), whereas common carp are omnivorous species repeatedly probing and scanning the substratum.

An important component of behaviour of aquatic animals are diel vertical and horizontal migrations. These are particular important in relatively deep standing waters but also occur in large slow-flowing rivers. In both vertical and horizontal migration, such behaviour is believed to reflect mainly the diurnal variation in habitat specific trade-offs between predation and food availability (Stich & Lampert, 1981; Lampert, 1989; Muška *et al.*, 2013). Particularly well-known phenomenon is the diel vertical migration of zooplankton; however, small fish and some benthopelagic crustaceans (including mysid shrimp such as the one studied in Chapter 3) show the same behaviour. The most common pattern of migration is ascend to surface layers with the onset of darkness and descend to deeper (and darker) regions around dawn (Lampert, 1989). For fishes in lakes and reservoirs, cyclic diel movements between the inshore and offshore zones are usual and may act on partial or population-wide levels depending on species and size classes (Muška *et al.*, 2013). During the day, larger fish are usually located in the offshore areas of reservoirs, whereas juvenile and small-sized fish avoid the offshore zone with a higher occurrence of predatory fish until dusk and move back to inshore at dawn (Jacobsen *et al.*, 2004; Draštík *et al.*, 2009, Muška *et al.*, 2013).

Seasonal movements and migration

Many fishes exhibit substantial seasonal variation in daily movement rates (Snedden *et al.*, 1999). Riverine fish were found to change habitat use and migrate mainly during the feeding, wintering and spawning period. Their seasonal movements may be generally characterised by upstream reproductive migration during spring (Koed *et al.*, 2000, Parkinson *et al.*, 1999) or autumn (Jonsson *et* *al.*, 2007), followed by downstream movement and summer activity associated with foraging (Lucas & Batley, 1996) and reduced activity in winter, with fish occupying suitable overwintering areas (Lucas & Batley, 1996; Baade & Fredrich, 1998). In riverine fishes, such seasonal movements are mainly influenced by the day length and water temperature (Lucas & Batley, 1996; Koed *et al.*, 2000; Geeraerts *et al.*, 2007).

The most pronouncing movements in riverine fishes are those relating to spawning (Bresser *et al.*, 1988; Aoyama, 1997), which can cover the entire length of the river (McCormick *et al.*, 1998). In fishes in particular, migratory behaviour and reproductive requirements are frequently tightly linked and are often further moderated by physiological adaptations. Extreme examples of these phenomena are reproductive migrations, strong home stream fidelity, and extensive migration-related changes in physiology exhibited by salmon or eel (Leggett, 1985; Palstra & van den Thillart, 2010). Regular habitat shifts connected with large-scale migration are also known for riverine cyprinids. For example, maximum distance of Prussian carp *Carassius auratus gibelio* spring migration reached 85 km and mostly stopped when fish reached an area where the reproduction takes place (Slavík & Bartoš, 2004). Annual change of habitats allows exploiting optimal conditions in oxbows or floodplain areas for spawning and growth of juvenile fish, and the use of feeding grounds rich in benthos in the main river by adults (Molls, 1999; Slavík & Bartoš, 2004).

Spawning migration has an adaptive character. Movements and migration tightly coupled to reliable environmental signals may greatly reduce the environmental variance actually experienced by the adults and their offspring (Leggett, 1985). For example, appropriate timing of the spawning migration, with respect to day-to-day fluctuations in flow and turbidity, may reduce predation risk, enhance access to spawning grounds in suitable conditions, and minimize energy losses for spawning in areas of strong water current (Keckeis, 2001; Rakowitz *et al.*, 2008). The right timing of spawning migration is usually triggered and maintained by a combination of environmental cues, mainly changes in water temperature, flow, lunar cycle or photoperiod (e. g. Jonsson, 1991; Buría *et al.*, 2007; Rakowitz *et al.*, 2008).

Turbidity

Turbidity imposes a considerable environmental constraint with a potential to affect whole fish communities. As a consequence of increased sediment loads turbidity increases occur under both natural and anthropogenic conditions; however, increasing levels of turbidity caused by eutrophication, agricultural runoff and other anthropogenic activities became a serious ecological concern worldwide (Collins *et al.*, 2009; Kemp *et al.*, 2011). Artificially elevated sediment loadings reduce the depth of the photic zone, thereby decreasing both primary and secondary production, with specific impacts including reduced growth and abundance of fish (Sigler *et al.*, 1984; Lloyd, 1987; Newcombe & Jensen, 1996) and significant declines in feeding rate, food assimilation and reproductive potential of benthic invertebrates (McCabe & O'Brien, 1983). Fish are directly affected by fine sediment either in suspension or deposited on the substrate and their biological response depends on suspended sediment concentrations and exposure durations (Newcombe & Jensen, 1996). These effects operate via influencing food availability and foraging efficiency, physiology and behaviour (Redding *et al.*, 1987; Kemp *et al.*, 2011; Michel *et al.*, 2013), however they are also suspected of modifying the movement and migration patterns of many fish species (Berg & Northcote, 1985; Lloyd *et al.*, 1987; Newcombe & MacDonald, 1991). For example, banded kokopu juveniles, *Galaxias fasciatus,* in the rivers of New Zealand did not enter turbid waterways during their upstream migration, which had serious implications for recruitment of this species in the particular catchment (Schicker *et al.*, 1990).

Turbidity alters the quality and quantity of visual information received by the fish; the effect is due to decreased light intensity as well as light scattering, the latter having more pronounced effects (Vinyard & O’Brien, 1976; Bruton, 1985; Hinshaw, 1985). Increased turbidity influences visually orienting fishes by deterioration of visual conditions, thus affecting their foraging efficiency by eliminating reactive distance at which visual predators can detect their prey (Vinyard & O’Brien, 1976). Not all of these effects are necessarily detrimental to aquatic organisms; for example, many fish and invertebrates thrive in turbid environments (Blaber & Blaber, 1980; Jeppesen *et al.*, 1994; Horppila *et al.*, 2004), presumably benefiting from a reduced risk of predation.

Nevertheless, sedimentation and turbidity can contribute towards decreases in fish populations since many fish simply relocate when ambient sediment loads are increased (Boubée *et al.*, 1997). If high suspended sediment loads persist, a general shift in community composition may ultimately occur, e.g. from salmonid to non-salmonid fish communities. This may reflect a higher tolerance to turbidity of groups such as the cyprinids (Gradall & Swenson, 1982) due to their ability to utilize plants and detritus as a food resource (Persson, 1983; Vinni *et al.*, 2000) and their large reproduction capacity and flexibility (Barthelmes, 1983). Cyprinids are also efficient foragers in low light intensities (Bergman, 1988), which may explain their relative success in the dim and turbid environments (Sandström & Karås, 2002).

River obstacles

River obstacles, and dams in particular, are recognised as serious factors affecting movement of fish in freshwater ecosystems. River obstacles interrupt ecological connectivity and limit dispersal of fish in both up- and downstream direction (Reyes-Gavilan *et al.*, 1996). Detrimental effect on riverine fishes is further magnified by associated river modifications to improve drainage or navigation and changes in natural flow regime leading to damage or loss of habitats associated with particular lifecycles stages (Balon *et al.*, 1986; O´Hara, 1993). Particularly, the availability of suitable nursery areas can be an important factor in determining the abundance of a fish population, since reproductive success and juvenile survival are major drivers of recruitment and thus limit the viability of populations (Mills & Mann, 1985). In many rivers, shallow, well-vegetated margins and back-waters are important nursery areas for larval and juvenile fishes and destruction of such nursery areas may lead to decline of fish populations (Mills & Mann, 1985; O´Hara, 1993; Scheidegger & Bain, 1995).

Besides, the negative impacts of river fragmentation on fish include reduced reproductive opportunities due to loss of habitats (Lucas & Batley, 1996; Bonner & Wilde, 2000), genetic deterioration of population (Morita & Yamamoto, 2002) or changes in fish community structure (Scheidegger & Bain, 1995; Slavík & Bartoš, 2001) which can finally lead to species decline or extinction (e.g. Axford, 1991; Morita & Yamamoto, 2002; Calles *et al.*, 2010). For example, changes in river volume and channel morphology in the Canadian River (New Mexico, Oklahoma, and Texas) were associated with an almost complete replacement of large-river species, which historically dominated the assemblage, by species that are characteristic of small tributary streams in the drainage (Bonner & Wilde, 2000).

Ongoing changes in riverine habitats associated with the invasion of new species, changing climate and improvement or deterioration of habitats influenced by anthropogenic activity such as eutrophication or migration barriers, motivate further studies on animal behaviour under natural conditions (Lucas & Baras, 2000). An individual confronted with changing environment has various biochemical, physiological, behavioural and morphological mechanisms that may, to a greater or lesser extent, buffer any adverse effects of change (Wootton, 1998). During my study, I have analysed behavioural reaction of several species (fish and a crustacean) across a range of environmental conditions in attempts to define the impacts of both natural changes and those caused by human activity.

2 Characteristics of studied species

Apart from evaluation of young-of-the-year fish assemblages, my thesis synthesize studies on three species inhabiting riverine environment – European grayling *Thymallus thymallus*, ide *Leuciscus idus* and an invasive crustacean *Hemimysis anomala*. To introduce them, I provide below a brief description of their biological characteristics and environmental requirements.

Ide – *Leuciscus idus* – Jelec jesen

The ide *Leuciscus idus* (Fig. 1) is a benthopelagic, riverine cyprinid inhabiting deeper, slower flowing reaches of lowland rivers. They are visually oriented feeders, predominantly consume insects. Ide may be distinguished from other species of *Leuciscus* in Europe by reddish tinge of all fins except dorsal. It typically reaches a size of about 450 mm SL, however individuals up to 850 mm SL were recorded (Kottelat & Freyhof, 2007). Juveniles are gregarious, adults more solitary. Ide is declining in numbers across Europe, classified as vulnerable by IUCN Red List criteria (2001) and protected as an endangered species in the Czech Republic (Lusk *et al.*, 2004).

Ide is a lithophytophilous spawner that requires running water for the eggs to develop (Balon, 1975) and spawns in March to April, when temperatures rise above 10°C. Females attach sticky eggs to gravel or submerged plants. Juveniles and feeding larvae inhabit a wide variety of shoreline habitats, which leave for deeper waters when growing. Juveniles reach up to 130 mm SL during first year (Kottelat & Freyhof, 2007).

Migratory pattern of ide has been described as potamodromous, performing upstream prespawning migrations in early spring followed by downstream movements directly after spawning. Migration occurs entirely in fresh water (Cala, 1970). Individual year-round movement patterns in ide may be very high; there are nearly resident individuals using a river stretch of only a few km for spawning, feeding and wintering, but also individuals that migrated for distances more than 100 km (Winter & Fredrich, 2003, Chapter I). In the River Elbe, ide were found mainly in the main channel of the river and connected backwaters, whereas during the spawning period, ide were found near river banks with submerged vegetation and relatively fast flow (Winter & Fredrich, 2003).

Fig. 1 Ide *Leuciscus idus*, caught under Svádov near Ústí nad Labem. Individual was tagged with radio transmitter with external antennae and later migrated downstream to German part of the River Elbe (photo provided by Pavel Horký).

European grayling – *Thymallus thymallus* – Lipan podhorní

The European grayling *Thymallus thymallus* (Fig. 2) belongs to the family Salmonidae (Wheeler, 1969). The distinguishing feature of the species is the presence of a large dorsal fin, which varies in size and shape between males and females, resulting in sexual dimorphism within this species (Witkovski *et al.*, 1984). Grayling is a typical rheophilic species that inhabits rivers and larger, rapidly flowing streams (Mallet *et al.*, 2000). The water quality requirements of grayling are similar to brown trout: cool, well oxygenated water with a sequence of pool, riffle, glide and run (Wooland, 1986). Although the environmental requirements are broader for grayling than brown trout, Wooland (1986) has stated that grayling succumb to pollution and higher temperatures more rapidly than brown trout. Historically, European grayling have occurred in high abundance, but their populations have declined due to habitat degradation, flow regulation for hydropower production, barriers to movement, or the introduction of non-native species (Persat, 1996; Mallet *et a*l.*,* 2000; Ovidio *et al.*, 2008).

Graylings typically shift habitat type with increasing size and age, and their distribution is related to speed of the current: the greater the fish size, the stronger the flow in the occupied stretch of stream may be (Sempeski *et al.*, 1997; Ingram *et al.*, 1999). Graylings tend to aggregate in schools outside the breeding season (Fabricius & Gustafson, 1955) and an important intraspecific segregation according to depth occurs: large individuals prefer deeper water than small ones (Sempeski & Gaudin 1995; Mallet *et al.*, 2000). Similarly, the type of prey consumed by graylings varies with age. In general, the consumption of benthic prey increases with age of graylings (Wooland, 1988), replacing the drifting invertebrates prevailing in the diet of juveniles that tend to stay close to the surface.

European grayling is a lithophilous spawner, which hides its brood under gravel (Balon, 1975). Graylings spawn in spring, usually from February to May, depending on annual variations of hydroclimatic conditions (Ovidio *et al.*, 2004). Outside the spawning period, graylings inhabit pool-riffle sequences, and usually stay in the stagnant waters during colder months. Distances travelled during the spawning migration varies within localities, usually reaching between 2-15 km (Ovidio *et al.*, 2004; Heggenes *et al.*, 2006). Spawners remained approximately 10 days at the spawning grounds and later performed a post-spawning homing to the pool-riffle sequences inhabited outside the spawning season (Ovidio *et al.*, 2004).

Fig. 2 European grayling *Thymallus thymallus*

Bloody-red Shrimp – *Hemimysis anomala* – vidlonožec

Hemimysis anomala (Fig. 3) is a necto-bentic mysid shrimp native to the Black and Caspian Seas (Pienimäki & Leppäkoski, 2004; Holdich *et al.*, 2006), which has become one of the invaders of the river systems of continental Europe (Wittmann, 2007). The first population of *H. anomala* outside of the Ponto-Caspian basin got established in 1962 in the Kaunass reservoirs in Lithuania after deliberate introduction to improve fish food supply. It subsequently spread into the Baltic Sea basin proper in 1992, the River Rhine basin in 1997, and United Kingdom in 2004 (Salemaa & Hietalahti, 1993; Ketelaars *et al.*, 1999; Holdich *et al.*, 2006). In the Czech Republic, it was first recorded in the River Elbe in 2003, six years after its first occurrence in the River Rhine (Horecký *et al.*, 2005).

H. anomala possesses several life history features that may facilitate its establishment in new habitats, including rapid growth and maturation. They occupy both lentic and lotic environments, including the margins of relatively fast-flowing rivers and tolerate a wide range of salinities (Borcherding *et al.*, 2006; Holdich *et al.*, 2006). They have an individual lifespan of about 9 months, mature in just 45 days, and can produce up to four generations per year. Clutch size varies with female size and season, ranging from 14 to 66 eggs (Ioffe, 1973).

H. anomala specimens have been usually found swarming around jetties, between boulders and under hard surfaces, particularly in the evening hours, and often undergo diurnal vertical and horizontal migrations (Holdich *et al.*, 2006). Adults are between 5.5 and 12.5 mm in length, and may be deep red to almost transparent with a touch of ivory-yellow colour (Komarova, 1991; Borcherding *et al.*, 2006; Holdich *et al.*, 2006). *H. anomala* is omnivorous; larger individuals prefer zooplankton, whereas smaller ones feed more on phytoplankton, the species thus may have an impact on both zooplankton and phytoplankton populations (Borcherding *et al.*, 2006). As *H. anomala* is a large-bodied, lipid-rich prey, it represents a high quality energy source (Borcherding *et al.*, 2006) and planktivorous fish may ultimately limit its abundance.

Fig. 3 Invasive nectobentic mysid shrimp *Hemimysis anomala* (photo provided by Jean-François Cart)

3 Outline of individual studies included in the thesis

Part I

The first part of the thesis is composed of three chapters (two published, and one in a manuscript form) that focus on movement behaviour of the three above-mentioned species in riverine environment. In all of these studies, I was responsible for analysis of the data, their interpretation, and wrote the manuscripts.

1 Factors influencing movement behaviour and home range size in ide *Leuciscus idus*

The first chapter included in my dissertation is an extensive radio-telemetry study, which examined behaviour of ide *Leuciscus idus*, in the River Elbe. Seventeen individuals of ide were tagged with radio transmitters and tracked from a boat weekly over periods of one year, from September 2003 to September 2004, to examine migration patterns and the influence of environmental factors on their diurnal behaviour and home range size. The study was carried out on the lowest part of the River Elbe in the Czech Republic, which nevertheless still represents the upper part of the whole river. The primary river stretch studied was c. 40 km long, from the dam at Střekov to the frontier with Germany. As the tagged fish were followed during spawning migrations, the studied stretch was extended as far as Meissen, Germany.

Ide occupied defined home ranges between which they relocated (e.g., during spring migration), with the mean home range size estimated to 19 500 ± 14 000 m^2. Our measurements indicated that the crucial factor influencing diurnal movement and the home range size of ide was season and turbidity. The ide activity increased as water temperature rose in the spring, resulting in greater movement and larger home ranges than during fall-winter period.

Running waters are currently experiencing a number of worsening anthropogenic stressors, including increasing sedimentation due to input of suspended matter (Kemp *et al.*, 2011). In habitats where vision serves as the primary source of information, reduced visual input from suspended sediment may lead to significant alterations in fish behaviour (Kemp *et al.*, 2011; Leahy *et al.*, 2011). In our study, we showed that ide extended their diurnal movements and home range size with increasing turbidity. Since elevated turbidity decreases the ability to obtain food (Sweka & Hartman, 2003), we suggested the increased activity of ide to be the result of increased foraging behaviour in turbid environment (Ferrari *et al.*, 2010).

The longest movements of ide occurred during the period of spawning activity in spring. Almost all individuals displayed downstream spring migrations, most of them remaining within Czech part of the River Elbe. Five individuals undertook longer migrations (68–100 km) to reach spawning sites near Dresden and Meissen in the German part of the river. The individuals that undertook the longest migrations started their run earliest, at the end of February. All final destinations of migration were shallow riffles with a gravel substratum, which provided suitable spawning and nursery habitats. Later in the season, the studied fish displayed homing behaviour and returned to within 0.5–2 km of their starting position. Previous reports on ide indicated an upstream pre-spawning migration followed by downstream movement after spawning (Cala, 1970), including studies in the middle reaches of the River Elbe (Winter & Fredrich, 2003). In our study, ide were observed to undertake similar long-distance migrations in spring but in the opposite direction, i.e. downstream during spring and returning upstream towards formerly occupied areas later in the season. Inconsistency in the direction of migration support the suggestion that ide is a flexible species capable of exhibiting a wide variety of movement patterns in multiple scales (Winter & Fredrich, 2003). Such behavioural plasticity exhibited in ide may enable this species to be less vulnerable to habitat fragmentation by barriers that is usually expected for potamodromous species.

2 Unlimited response to environmental factors: a telemetry study on the behaviour of wild and hatchery-reared European grayling *Thymallus thymallus*.

In the last decades, populations of European grayling, *Thymallus thymallus* (L.) decreased, being affected mainly by the loss or degradation of suitable habitats (Mallet *et al.*, 2000), overfishing (Näslund *et al.*, 2005) and migration barriers (Persat *et al.*, 1996). To support wild populations, stocking is usually carried out, although it represents a significant risk for the conservation of wild populations (Cowx, 1994; Persat, 1996; Bohlin *et al.*, 2002). Artificial rearing and subsequent stocking substantially influence fish behavioural traits, as they adapt during ontogenesis in hatchery to strikingly different environment in comparison to their wild conspecific (Jensen *et al.*, 1986; Huntingford, 2004). How cultured fishes behave may also determine the impact of farm escapees on natural fish stocks and therefore it is important to understand (Huntingford, 2004). The objectives of the presented study were to evaluate differences in post-stocking movement of wild and hatchery-reared European grayling and to find out which environmental factors influence their behaviour and how. Grayling home range size, diel movements and longitudinal movements were analysed. In three consecutive years, 40 juvenile grayling were radio-tracked during autumn-winter period in the River Blanice, Czech Republic.

Both wild and hatchery-reared grayling increased their diel movements and home range size according to increasing light intensity, flow, temperature, and turbidity, but the character of their response differed. In general, response of wild fish to environmental variability had a threshold-related character. Wild fish usually responded to stimuli only up to/from a certain level and the effect of further increase/decrease of measured variable on their behaviour levelled out, whereas hatchery reared fish responded with continuous increase in intensity of observed behaviour. The un-terminated behavioural response of the hatchery-reared grayling could be viewed as a result of lack of juvenile experience from the river (Jonsson *et al.,* 1990), and thus lack of opportunity to learn favourable abilities (Vehanen *et al.*, 2009). The diel movements of grayling ranged from 0 to 98 m; home range size from 21 to 1428 m^2 and longitudinal movements from 0 to 4125 m. Hatchery-reared fish displayed larger total migration length over the study period than wild fish, which was caused mainly by their dispersal in the downstream direction, most probably as a consequence of adaptation to local environments and competition for feeding and/or sheltering sites with the resident fish (Deverill *et al.*, 1999; Sundström & Johnsson, 2001; Huntingford, 2004).

Hatchery-reared fish were mostly active during daytime, in contrast to wild fish with more intense nocturnal movements. Different diel patterns of activity in hatchery fish in comparison to their wild counterparts may be generated by experience in rearing environment which shapes their behaviour (Olla *et al.*, 1998; Bateson & Martin, 1999; Álvárez & Nicieza, 2003). Hatchery fish grow up in different conditions than wild ones; in some respects, the hatchery environment is less challenging as the food is readily available and fish are protected against predators (Huntingford, 2004). Daytime activity in hatchery grayling might therefore arise as the fishes habituate to the timing of feeding in the hatchery, and a lack of predation pressure. Nocturnal activity in wild fish may however be related also to seasonal changes in behaviour. Graylings in our study were observed during autumn-winter period, which is often reported to be associated with night time activity in salmonids (Heggenes *et al.*, 1993; Valdimarsson & Metcalfe, 1998; Fraser *et al.*, 2003). This reflects a temperature-dependent shift in diel activity related to minimizing the mortality costs of foraging (Fraser *et al.*, 1993; Metcalfe *et al.*, 1999). Overall, the influence of hatchery environment on grayling behaviour in the wild was obvious; therefore we suggest that the habitat restoration and natural spawning renewal should be of the primary importance to conserve indigenous grayling populations.

3 Diurnal behavioural patterns and spread of the Ponto-Caspian invader *Hemimysis anomala* in the Elbe River

Data for the third study assessing the spread and diel behaviour of freshwater crustacean *Hemimysis anomala* at the River Elbe arose when the first specimen of *Hemimysis anomala* was found during examining invertebrate drift as a food resource for fish. As this was, after the first record of Horecký *et al.* (2005), the second record of this crustacean in the Czech Republic, and only a few studies focused on this invasive amphipod in rivers, we decided to analyse its diurnal behaviour and to investigate its further spread. To evaluate the presence/absence of *H. anomala*, we used the data collected for regular sampling of macroinvertebrate community structure which were sampled from 2006 to 2008 at 896 sites scattered across the whole Czech Republic. Diurnal behaviour of *H. anomala* was assessed using drift net sampling from July to September 2007 in the Czech part of the River Elbe.

H. anomala appears to be spreading progressively through the Elbe. By 2008, *H. anomala* was recorded throughout a 129 km long stretch of the Elbe from the border with Germany to just north of the confluence with the Vltava River, the species was however not found on other sites surveyed. The records of *H. anomala* in the lower section of the Elbe, which is characterized by the presence of harbours and frequent shipping, support the suggestion that this species is dispersed largely via shipping (Wittmann, 2007).

When studying *H. anomala* behaviour, a total of 180 drift net samples were collected during ten 24-h cycles in 2007 in the river Elbe near Ústí nad Labem. Densities of drifting *H. anomala* varied from 0 to 162.8 ind m^{-3} comprising on average 36% males, 13% females and 51% juveniles, indicating that the species is reproducing in the river Elbe. Analyses of drift density of *H. anomala* at different depths revealed that it occupied the mid-water layer of the water column, with significantly more individuals in the bottom and mid-water than the surface. The density and the length of drifting individuals increased with decreasing light levels and increasing turbidity. This aspect is probably an outcome of two major factors: predator avoidance and increased resource availability. Decreased light levels and/or increased turbidity may offer shelter for mysids, by reducing the feeding success of fish (Kirk, 1981).

Overall, we have shown that drifting may facilitate further range extension of this invasive amphipod (Van Riel *et al.*, 2011), whose dispersal may be underestimated by common monitoring practices.

Part II

The second part of my thesis comprises two closely linked chapters (both published) that focus on composition of young-of-the-year (YOY) fish assemblages in Czech rivers. I have contributed to these studies by collecting and analysing the YOY samples as well as by part of data analysis and part of the text in the Chapter 4. These results are used for comparison of different methodologies for assessing ecological quality of running waters and to evaluate the effect of river obstacles on YOY composition.

4 Young-of-the-year (YOY) assemblage sampling as a tool for assessing the ecological quality of running waters

The study presents the method to classify ecological quality of waters for the requirements of the Water Framework Directive (WFD; 2000/60/ECC) using the young-of-the-year (YOY) fish assemblages. The Water Framework Directive requirements are to classify ecological quality of waters using different biological quality elements. Fish represents one of these elements, being usually assessed in running waters by unified European Fish Index (EFI; Schmutz *et al.*, 2007) with the methodology based on the sampling of the fish assemblage with emphasis on the adults. The aim of the study was to test whether sampling of YOY fish is applicable for assessing the ecological quality in running waters as well. To address this issue, the relationship between EFI values estimated with YOY and adult fish was described, based on collection of samples from 55 river sites scattered across the Czech Republic. Moreover, seven sites were sampled annually from 1995 to 2006 along the longitudinal profile of the Elbe River to evaluate long-term changes in fish community structure.

Although EFI values estimated with YOY and adults are positively correlated, indicating that YOY and adult sampling is comparable, YOY bias EFI towards lower values and classify higher number of sites into the poor and bad ecological status. Long-term YOY data from the River Elbe showed that the number of the successfully reproducing species significantly decreased from 1995 to 2006. In contrast, the number of species in adult fish assemblage did not show any significant trends, most probably reflecting the regular stocking from the Czech Anglers Union. Overall, YOY sampling represents useful method for assessing ecological function and integrity of riverine systems. The advantage of this method is that it sensitively reflects species-specific life-history (Kryzhanovsky, 1949), availability of spawning/nursery habitats (Oberdorff & Hughes, 1991; Copp, 1992) and provide a proper response to population dynamics (Balon, 1984) with lower costs than the sampling of adults.

5 The response of the young of the year fish to river obstacles: Functional and numerical linkages between dams, weirs, fish habitat guilds and biotic integrity across large spatial scale.

The fifth chapter of my thesis focuses on response of fish community to river obstacles. In our study, we attempted to identify relationships between river obstacles and fish community structure to either support or reject the hypothesis that fish communities will show responses to obstacles at a river scale. In order to find out how river obstacles influence fish community structure, we assessed young of the year (YOY) fish of 35 fish species from a total of 54 study sites, including rivers ranging in size (from small to large) and anthropogenic impact (from those that are pristine to greatly affected by human activities).

Fish are an important indicator for assessing the ecological integrity of rivers (Karr, 1991). Their value as indicators results from the broad range of habitat used during their life cycle, from bottom sediment for egg and larval development to the longitudinal integrity required, e.g., for spawning migrations (Persat *et al.*, 1994). We chose to focus on juvenile (YOY) fish, because obstacles have been shown to affect the reproductive success and juvenile survival of riverine fish populations (Schlosser, 1985; Copp *et al.*, 1991). We have investigated the effects of the number, type and spatial placement of obstacles relative to the scale of the river. The study sites were distributed across the hydrological network of the Czech Republic. Two types of obstacles (1118 weirs and 28 dams for a total of 2126 obstacles) were considered, for which numerical and distance-related descriptors were derived using a geographic information system (GIS). To analyse fish community structure independently of natural longitudinal zonation (Sheldon, 1968; Vannote *et al.*, 1980), we expressed the YOY fish community structure as the probability of habitat-based functional-guild dominance (Oberdorff *et al.*, 2001, 2002; Pont *et al.*, 2006), considering fish species-specific habitat requirements. Among them, rheophillic species with all freshwater life stages linked to lotic environment, and eurytopic species, tolerant to either lotic or lentic environments were covered.

Among the most frequently encountered rheophilic species were *Gobio gobio*, dace *Leuciscus leuciscus*, stone loach *Barbatula barbatula* and ide, *Leuciscus idus*. The most frequently recorded eurytopic species included bleak, *Alburnus alburnus*, roach *Rutilus rutilus* and perch *Perca fluviatilis*. We have shown that all the obstacles considered together were found to be the most relevant predictor of YOY fish community structure response. The negative impact on YOY fish (a loss of rheophilic species) increased with the increasing number of obstacles and the decreasing relative distance between two consecutive obstacles. A high probability of dominance of eurytopic species indicated alteration in riverine ecosystems and suggested a more degraded fish community structures at a particular site.

Appendix

Validation of scale-age determination in European grayling *Thymallus thymallus* using tag-recapture analysis

Accurate determination of fish age is essential for fisheries management as it provides an effective tool for assessing growth parameters and the production of fish populations, which in turn are important for fish stock assessment and sustainable exploitation rates. Incorrect estimation of fish age using scales as a method of age determination has been reported in previous studies (McBride *et al.*, 2005; Maceina & Sammons, 2006). The objective of the study was to provide a validation of accuracy of scale-based age estimates in *T. thymallus* which was not available in previous studies. The analysis was based on 3997 individually tagged *T. thymallus* caught in the River Wylye, a spring-fed chalk stream draining into the River Avon, south-west England. The fish were collected using semi-quantitative electrofishing each October for eight consecutive years (1999–2007) and tagged with VI tags and passive integrated transponder (PIT) tags. The fish were sampled for scales at tagging and then again at recapture and the fork length (LF) of each fish was measured. Using long-term mark–recapture data, the real ages of fish of all age categories were provided.

The analysis indicated significant and increasing deviation between scale-read age and tag age for older individuals. Agreement with tag-recapture (true) age was highest in 1+ and 2+ year fish, with

4.15% of incorrect scale readings. The percentage of incorrect scale readings increased in older age classes. There was a tendency to underestimated the age of fish aged 4+ years; the age of most fish aged ≥5+ years was incorrectly assigned using scales. Ages were incorrectly assigned to all fish that were aged 7+ years, the oldest age class encountered in the study.

The reported maximum ages of *T. thymallus* are highly variable and dependent on geographical location and environmental conditions. Reports from waters of Britain suggest that *T. thymallus* die by their fifth or sixth year (Crundwell, 1991; Davies *et al.*, 2004), and similar ages were reported from Czech rivers, with the maximum recorded age of 3 years (Nagy, 1984) and 5 years (Lusk, 1975). Nevertheless, it is not clear whether any of these estimates of longevity are accurate. The present results revealed that maximum ages based on scales readings are underestimates and that *T. thymallus* live longer than indicated in previous studies. Our findings are in more accordance with the results of Woolland (1987), who reported a maximum age of 6–7 years from the more upland River Dee catchment and Llyn Tegid, Wales. Such results also suggest that the rate of survival of the *T. thymallus* population studied is higher than would be predicted from scale-age determination. Hence, the population would have a corresponding lower growth potential than expected from scale readings. Such an underestimate could lead to more exhaustive exploitation of the population on the assumption that renewal of the population would occur at a faster rate.

4 Conclusion and future prospects

There is a growing awareness that the river management practices need to become more sensitive to the needs for migration and dispersal by freshwater animals. Many species have been affected by changes in the water management that reduced spawning and rearing areas and reduced the area over which migration can occur, although this has a substantial importance for their lifetime reproductive success and population dynamics in rivers. The extent of impact of environmental variation on individual species nevertheless often remains unknown. Application of advanced techniques such as telemetry may open new possibilities, and thus improve our abilities to understand the nature and significance of animal movement and migration. In my thesis, I have made an effort to enlighten at least a few of the many unanswered questions relevant in this context.

Observed behaviour of the studied riverine species indicated that they adapt to changing environment by variable extent of movement and migration. Studies of the movement behaviour of ide and grayling bring new insights on the movement flexibility of these fishes, whose behaviour has not been analysed in detail so far. Both ide and grayling occupied defined home ranges but were also able to relocate in response to various stimuli of the river environment. The study on ide (**Chapter I**) revealed the extensive range of their migration and home fidelity. Ide occupied defined home range during the year, however, in spring they undertook migration up to 100 km to reach suitable spawning areas and later in the season returned back to vicinity of their original home range sites. Radio-tracking of ide migration also showed unusual downstream direction of migration routes, most probably in response to river obstacles. Such adaptation suggests great plasticity in movement behaviour for this species. Variations in movement behaviour among grayling of wild and hatchery origin were most evident in activity peaks in response to environmental influences which were unterminated in hatchery-reared grayling (**Chapter II**). We concluded that modified behavioural patterns in hatchery-reared fish are associated with different rearing environments. Artificial culture conditions relax the selective pressures from predators and impose new selection that adapts fish to confinement (Huntingford, 2004). However, it is not clear which factors in the hatchery most strongly impacted fish behaviour after release, and pinpointing these should be the aim of further investigation. Various aspects shaping the behaviour should also be considered when attempting to predict the consequences of release or escape of domesticated animals in the wild.

Various environmental factors affected movement of observed animals; however, the common feature of the observed effects of environmental factors on animal behaviour was the effect of water turbidity. Although we expected that this environmental factor would be substantial, originally we did not assume that it would play such an essential role, manifested in behaviour of all studied species. Both ide and grayling extended their diel movements and home range size with increasing turbidity and also the occurrence of *Hemimysis anomala* in drift (**Chapter III**) was substantially influenced by turbidity levels. Behavioural response may be attributed primarily to altered predator-prey interactions due to reduced visibility – increased activity is most probably the result of enhanced foraging behaviour in turbid environment and protection from predators (Gregory & Northcote, 1993; Bonner & Wilde 2002; Ferrari *et al.*, 2010). Although my studies have shed some light on the behaviour of observed species, further work is required to improve our understanding on interactions of environmental and biotic factors. Further research should further elucidate how turbidity influence behavioural interactions, both between fish and their invertebrate prey and between predator and prey fish.

Behaviour and life cycles of animals in running waters are remarkably influenced by changes in environment arising from anthropogenic disturbances. To classify ecological quality of waters according the Water Framework Directive (WFD; 2000/60/ECC) requirements, variable tools are used and fish community structure represents one of them. In **Chapter IV,** we tested whether the YOY assemblage sampling is relevant for assessing the ecological quality of running waters in relation to European Fish Index (EFI), a method based on evaluation of adult fish assemblages (Schmutz *et al.*, 2007). YOY sampling was proved to be effective method due to its ability to reflect sensitively responses to the habitat structure regardless of the effect of stocking or river size. This study also revealed that the number of reproducing species significantly decreased in the river Elbe in the last decades. Dramatic declines in some species of freshwater fishes in river systems have been at least partly due to blockage of migration routes and damage to habitats associated with particular lifecycle stages.

Movement is an important adaptive trait allowing to cope with environmental variability, and obstacles limit the mobility of water animals and their ability to avoid or to adapt to changes they have to face. Analyses of density of river obstacles across the hydrological network (**Chapter V**) revealed that rivers with higher number of obstacles and the decreasing distances between them had a lower abundance of YOY fishes and that the juvenile assemblage contained a lower proportion of typical riverine rheophilic species. Fish utilize various environments within river system and the movement and/or migration between habitats essential for feeding, spawning or nursery habitats are of utmost importance for their survival and reproduction. Impassable obstacles therefore have far-reaching consequences for the life cycle of individual fish as well as dynamics of their populations. Observed changes in fish community structure indicated that both weirs and dams substantially modify local habitats, influencing the suitability of environment to particular species. YOY assemblages were dominated by cyprinids, thanks to their ability to adapt their behaviour to the habitat fragmentation by completing their life cycles in environment limited by physical barriers (Lucas & Baras, 2001; Geeraerts *et al.*, 2007). Another serious impact caused by river obstacles is a "barrier effect", disrupting or limiting the migration of organisms throughout the river system (Pringle, 2001; Fagan, 2002). Therefore, consideration should be given to ways of mitigating effects of existing barriers.

There is a need for appropriate fish passage facilities which would enable unrestricted movement for both juvenile and adult fish. They would allow fish to adapt to changing environmental conditions, access essential habitats to meet life-history needs and promote the recovery of fish populations in rivers.

In the study evaluating the accuracy of the scale-read age in European grayling (**Appendix**), we have shown that this method provides good results for fish up to 4+ years old and that the maximum age of grayling are highly variable and dependent on geographical location and environmental conditions. Although this study provided only elementary results, the valuable dataset provided should become a basis for further study on growth parameters and population structure of grayling in the River Wylye. Furthermore, proper assessment of true age for European grayling may improve the management of its populations throughout the species' range.

5 References

Álvarez, D. & Nicieza, A. G. (2003). Predator avoidance behaviour in wild and hatchery reared brown trout: the role of experience and domestication. *Journal of Fish Biology* **63**, 1565–1577.

Aoyama, T. (1997). Long distance movements of anadromous whitespotted charr (*Salvelinus leucomaenis*) in southern Hokkaido, Japan. *Scientific Report of Hokkaido Fish Hatchery* **51**, 63–65.

Axford, S. N. (1991). Some factors affecting catches in Yorkshire rivers. Rehabilitation of Freskvater Fisheries (ed. I.G. Cowx), pp. 143–153. Blackwell Scientific Publications, Oxford.

Baade, U. & Fredrich, F. (1998). Movement pattern of activity of the roach in the River Spree, Germany. *Journal of Fish Biology* **52**, 1165–1174.

Balon, E. G. (1975). Reproductive guilds of fish: a proposal and definition. *Canadian Journal of Fisheries and Aquatic Sciences* **32**, 821–864.

Balon, E. K. (1984). Reflections on some decisive events in the early life of fishes. *Transactions of the American Fisheries Society* **113**, 178–185.

Balon, E.K., Crawford, S. S. & Lelek, A. (1986). Fish communities of the upper Danube (Germany, Austria) prior to the new Rhein-Main-Donau connection. *Environmental Biology of Fishes* **15**, 243–271.

Barthelmes, D. (1983). Effects of eutrophication and fisheries management on fish faunas of cyprinid lakes. *Roczniki Nauk Rolniczych, Series H* **100**, 31–44.

Bateson, P. & Martin, P. (1999). Design for a Life: How Behaviour Develops. London:Jonathan Cape.

Bauer, Ch. & Schlott, G. (2004). Overwintering of farmed common carp (*Cyprinus carpio* L.) in the ponds of a central European aquaculture facility – measurement of activity by radio telemetry. *Aquaculture* **241**, 301–317.

Berg, L. & Northcote, T. G. (1985). Changes in territorial gill flaring, and feeding behaviour in juvenile coho salmon (*Oncorhynchus kisutch*) following short-term pulses of suspended sediment. *Canadian Journal of Fisheries and Aquatic Sciences* **42**, 1410–1417.

Bergman, E. (1988). Foraging abilities and niche breadths of two percids, *Perca fluviatilis* and *Gymnocephalus cernua*, under different environmental conditions. *Journal of Animal Ecology* **572**, 443–453.

Blaber, S. J. M., & Blaber, T. G. (1980). Factors affecting the distribution of juvenile estuarine and inshore fish. *Journal of Fish Biology* **17**, 143–162.

Bohlin, T., Sundström, L. F., Johnsson, J. I., Höjesjö, J. & Pettersson, J. (2002). Density-dependent growth in brown trout: effects of introducing wild and hatchery fish. *Journal of Animal Ecology* **71**, 683–692.

Bonner, T. H. & Wilde, G. R. (2000). Changes in the Canadian River fish assemblage associated with reservoir construction. *Journal of Freshwater Ecology* **15**, 189–198.

Borcherding, J., Murawski, S. & Arndt, H. (2006). Population ecology, vertical migration and feeding of the Ponto-Caspian invader *Hemimysis anomala* in a gravel-pit lake connected to the River Rhine. *Freshwater Biology* **51**, 2376–2387.

Boubée, J. A. T., Dean, T. L., West, D. W. & Barrier, R. F. G. (1997). Avoidance of suspended sediment by the juvenile migratory stage of six New Zealand native fish species. *New Zealand Journal of Marine and Freshwater Research* **31**, 61–69.

Bresser, S. W., Stearns, F. D., Smith, M. W., West R. L. & Reynolds, J. B. (1988). Observations of movements and habitat preferences of burbot in an Alaskan glacial river system. *Transactions of the American Fisheries Society* **117**, 506–509.

Bruton, M. N. (1985). The effects of suspensoids on fish. *Hydrobiologia* **125**, 221–241.

Buría, L., Walde, S. J., Battini, M., Macchi, P. J., Alonso, M., Ruzzante, D. E. & Cussac, V. E., (2007). Movement of a South American perch Percichthys trucha in a mountain Patagonian lake during spawing and prespawning periods. *Journal of Fish Biology* **70**, 215–230.

Burt, W. H. (1943). Territoriality and home range concepts as applied to mammals. *Journal of Mammalogy* **24**, 346–352.

Cala, P. (1970). On the ecology of ide *Idus idus* (L.) in the River Kävlingeån, South Sweden. *Reports of the Institute of Freshwater Research Drottningholm* **50**, 45–99.

Calles, O., Olsson, I. C., Comoglio, C., Kemp, P. S., Blunden, L., Schmitz, M. & Greenberg, L. A. (2010). Size-dependent mortality of migratory silver eels at a hydropower plant, and implications for escapement to the sea. *Freshwater Biology* **55**, 2167–2180.

Collins A. L., Anthony S. G., Hawley, J. & Turner T. (2009). Predicting potential change in agricultural sediment inputs to rivers across England and Wales by 2015. *Marine and Freshwater Research* **60**, 626–637.

Copp, G. H. (1992). An empirical model for predicting the microhabitat of 0+ juveniles in lowland streams. *Oecologia* **91**, 338–345.

Copp, G. H., Oliver, J. M., Peňáz, M. & Roux, A. L. (1991). Juvenile fishes as functional describers of fluvial ecosystem dynamics: applications on the Rhone, France. *Regulated River* **6**, 135–145.

Cowx, I. G. (1994). Stocking strategies. *Fisheries Management and Ecology* **1**, 15–30.

Crundwell, C. R. (1991). A study of the Grayling, *Thymallus thymallus* on the River Test at Leckford and Longparish, Hampshire, England. Diploma Thesis, Sparsholt College, Hampshire, UK.

Daugherty, D. J. & Sutton, T. M. (2005). Seasonal movement patterns, habitat use, and home range of flathead catfish in the Lower St. Joseph River, Michigan. *North American Journal of Fisheries Management* **25**, 256–269.

Davies, C. E., Shelley, J., Harding, P. T., McLean, I. F. G., Gardiner, R. & Peirson, G. (2004). Freshwater Fishes in Britain the Species and Their Distribution. Colchester: Harley Books.

Deverill, J. I., Adams, C. E. & Bean, C. W. (1999). Prior residence, aggression and territory acquisition in hatchery-reared and wild brown trout. *Journal of Fish Biology* **55**, 868–875.

Draštík, V., Kubečka, J., Čech, M., Frouzová, J., Říha, M., Jůza, T., Tušer, M., Jarolím, O., Prchalová, M., Peterka, J., Vašek, M., Kratochvíl, M., Matěna, J. & Mrkvička, T. (2009). Hydroacoustic fish stock estimates in temperate reservoirs: day or night surveys? *Aquatic Living Resources* **22**, 69–77.

Fabricius, E. & Gustafson, K. J. (1955) Observations on the spawning behaviour of the Grayling, *Thymallus thymallus* (L.). *Reports of the Drottningholm Freshwater Research Institute* **36**, 75–103.

Fagan, W.F. (2002). Connectivity, fragmentation, and extinction risk in dendritic metapopulations. *Ecology* **83**, 3243–3249.

Ferrari, M. C. O., Lysak, K. R. & Chivers, D. P. (2010). Turbidity as an ecological constraint on learned predator recognition and generalization in a prey fish. *Animal Behaviour* **79**, 515–519.

Fraser, N.H.C., Metcalfe, N.B. & Thorpe, J.E. (1993). Temperature-dependent switch between diurnal and nocturnal foraging in salmon. *Proceedings of the Royal Society of London* B **252**, 135–139.

Geeraerts, C., Ovidio, M., Verbiest, H., Buysse, D., Coeck, J., Belpaire, C. & Philippart, J.-C. (2007). Mobility of individual roach *Rutilus rutilus* (L.) in three weir-fragmented Belgian rivers. *Hydrobiologia* **582**, 143–153.

Gerking, S. D. (1953). Evidence for the concepts of home range and territory in stream fishes. *Ecology* **34**, 347–365.

Gowan, C., Young, M. K., Faush, K. & Riley, S. (1994). Restricted movements in resident stream salmonids: a paradigm lost? *Canadian Journal of Fisheries and Aquatic Sciences* **51**, 2626–2637.

Gradall, K. S. & Swenson, W. A. (1982). Responses of brook trout and creek chub to turbidity. *Transactions of the American Fisheries Society* **111**, 392–395.

Grant, J. W. A., Steingrímsson, S. Ö., Keeley, E. R. & Cunjak, R. A. (1998). Implications of territory size for the measurement and prediction of salmonid abundance in streams. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 181–190.

Gregory, R. S. & Northcote, T. G. (1993). Surface, planktonic, and benthic foraging by juvenile chinook salmon (*Oncorhynchus tschawytscha*) in turbid laboratory conditions. *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 233–240.

Hansen, E. A. & Closs, G. P. (2005). Diel activity and home range size in relation to food supply in a drift-feeding stream fish. *Behavioral Ecology* **16**, 640–648.

Harestad, A. S. & Bunnel, F. L. (1979). Home range and body weight – a re-evaluation. *Ecology* **60**, 389–402.

Heggenes, J., Krog, O. M .W., Lindas, O.R., Dokk, J.G. & Bremnes, T. (1993). Homeostatic behavioural responses in a changing environment: brown trout (*Salmo trutta*) become nocturnal during winter. *Journal of Animal Ecology* **62**, 295–308.

Heggenes, J., Qvenild, T., Stamford, M. D. & Taylor, E. B. (2006). Genetic structure in relation to movements in wild European grayling (*Thymallus thymallus*) in three Norwegian river. *Canadian Journal of Fisheries and Aquatic Sciences* **63**, 1309–1319.

Hinshaw, J. M. (1985). Effects of illumination and prey contrast on survival and growth of larval yellow perch *Perca flavescens*. *Transactions of the American Fisheries Society* **114**, 540–545.

Hixon, M. A. (1987). Territory area as a determinant of mating systems. *American Zoologist* **27**, 229–247.

Holdich, D., Gallagher, S., Rippon, L., Harding, P. & Stubbington, R. (2006). The invasive Ponto-Caspian mysid, *Hemimysis anomala*, reaches the UK. *Aquatic Invasions* **1**, 4–6.

Horecký, J., Šporka, F. & Stuchlík, E. (2005). First record of *Hemimysis anomala* SARS (Crustacea: Mysidacea) from Czech stretch of Elbe river (Czech Republic). *Lauterbornia* **55**, 89–91.

Horký, P., Slavík, O., Bartoš, L., Kolářová, J. & Randák, T. (2007*a*): Behavioural pattern in cyprinid fish below a weir as detected by radio telemetry. *Journal of Applied Ichthyology* **23**, 679–683.

Horký, P., Slavík, O., Bartoš, L., Kolářová, J. & Randák, T. (2007*b*). Docksides as a winter habitats of chub and pikeperch in the channelised Elbe River. *Fundamental and Applied Limnology* **168/3**, 281–287.

Horppila, J., Liljendahl-Nurminen, A. & Malinen, T. (2004). Effects of clay turbidity and light on the predator–prey interaction between smelts and chaoborids. *Canadian Journal of Fisheries and Aquatic* **61**, 1862–1870.

Huntingford, F. A. (2004). Implications of domestication and rearing conditions for the behaviour of cultivated fishes. *Journal of Fish Biology* **65** (Supplement A), 122–142.

Ingram A., Ibbotson, A. & Gallagher, M. (1999). The ecology and management of the European grayling *Thymallus thymallus* (Linnaeus). *Interim reports of the Institute of Freshwater Ecology*, 1–84.

Ioffe, T.s.I. (1973). Pool for acclimatization of invertebrates in the USSR. *Izv. Gos. Nauchno-Issled. Inst. Ozern. Rechn. Rybn. Khoz.* **84**, 18–68.

Jacobsen, L., Berg, S., Jepsen, N. & Skov, C. (2004). Does roach behaviour differ between shallow lakes of different environmental state? *Journal of Fish Biology* **65**, 135–147.

Jensen, A. J., Heggberget, T. G. & Johnsen, B. O. (1986). Upstream migration of adult Atlantic salmon, *Salmo salar* L., in the River Vefsna, northern Norway. *Journal of Fish Biology* **26**, 459–465.

Jeppesen, E., Sondergaard, M., Kastrup, E., Petersen, B., Hammershoj, R. B., Mortensen, E., Jensen J.P. & Have, A. (1994). Does the impact of nutrients on the biological structure and function of brackish and freshwater lakes differ? *Hydrobiologia* **276**, 15–30.

Jewell, P. A. (1966). The concept of home range in mammals. *Symposium of the Zoological Society of London* **18**, 85–109.

Jones, M. J. & Stuart, I. G. (2007). Movements and habitat use of common carp (*Cyprinus carpio*) and Murray cod (*Maccullochella peeli peeli*) juveniles in a large lowland Australian river. *Ecology of Freshwater Fish* **16**, 210–220.

Jonsson, B., Jonsson N. & Hansen, L. P. (2007). Factors affecting river entry of adult Atlantic salmon in a small river. *Journal of Fish Biology* **71**, 943–956.

Jonsson, B., Jonsson, N. & Hansen, L. P. (1990). Does juvenile experience affect migration and spawning of adult Atlantic salmon? *Behavioral Ecology and Sociobiology* **26**, 225–230.

Jonsson, N. (1991). Influence of water flow, water temperature and light on fish migration in rivers. *Nordic Journal of Freshwater Research* **66**, 20–35.

Karr, J. R. (1991). Biological integrity: a long neglected aspect of water resource management. *Ecological Applications* **1**, 66–84.

Keckeis, H. (2001). Influence of river morphology and current velocity conditions on the spawning site selection of *Chondrostoma nasus* (L.). *Archiv für Hydrobiolologie*, Supplement **135**, 341–356.

Kemp, P., Sear, D., Collins, A., Naden, P. & Jones, I. (2011). The impacts of fine sediment on riverine fish. *Hydrological processes* **25**, 1800–1821.

Ketelaars H.A.M., Lambregts-van de Clundert F.E., Carpentier C.J., Wagenvoort A.J. & Hoogenboezem W. (1999). Ecological effects of the mass occurrence of the Ponto-Caspian invader, *Hemimysis anomala* G.O. Sars, 1907 (Crustacea: Mysidacea), in a freshwater storage reservoir in the Netherlands, with notes on its autecology and new records. *Hydrobiologia* **394**, 233–248.

Kirk, J. T. O. (1981). Estimation of the scattering coefficient of natural waters using underwater irradiance measurements. *Aust. J. Mar. Freshw. Res*. **32**, 533–539.

Koed, A., Mejlhede, P., Balleby K. & Aarestrup, K. (2000). Annual movement and migration of adult pikeperch in a lowland river. *Journal of Fish Biology* **57**, 1266–1279.

Komarova, T. I. (1991). Fauna of Ukraine. Vol. 26. Mysidacea. *Naukova Dumka*, Kiev.

Kottelat, M. & Freyhof, J. (2007). Handbook of European freshwater fishes. Publications Kottelat, Cornol, Switzerland. 646 p.

Kryzhanovsky, S. G. (1949). Eco-morphological principles and patterns of development among minnows, loaches and catfishes. Part II: Ecological groups of fishes and patterns of their distribution. Akad Nauk SSSR, Tr Inst Morf Zhitovr im Akad AN Severtsova **1**, 237–231

Lampert, W. (1989). The adaptive significance of diel vertical migration of zooplankton. *Functional Ecology* **3**, 21–27.

Leahy, S. M., McCormick, M. I., Mitchell, M. D. & Ferrari, M. C. O., (2011). To fear or to feed: the effects of turbidity on perception of risk by a marine fish. *Biology Letters* **7**, 811–813.

Leggett, W. C. (1985). The role of migrations in the life history evolution of fish. In: Migration: Mechanisms and Adaptive Significance (M. A. Rankin, ed.), pp. 277–295. University of Texas Marine Science Institute, Port Aransas,TX.

Lloyd, D. S. (1987). Turbidity as a water quality standard for salmonid habitats in Alaska. *North American Journal of Fisheries Management* **7**, 34–45.

Lucas M.C. & Baras E. (2001). Migration of freshwater fishes. Blackwell Science, Oxford.

Lucas, M. C. & Baras, E. (2000). Methods for studying spatial behaviour of freshwater fishes in the natural enviroment. *Fish and Fisheries* **1**, 283–316.

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

Lusk, S. (1975). Distribution and growth rate of grayling (*Thymallus thymallus*) in the drainage area of the Svratka River. *Zoologické Listy* **24**, 385–399.

Lusk, S., Hanel, L. & Lusková , V. (2004). Red List of the ichthyofauna of the Czech Republic: development and present status. *Folia Zoologica* **53**, 215–226.

Maceina, M. J. & Sammons, S. M. (2006). An evaluation of different structures to age freshwater fish from a northeastern US rivers. *Fisheries Management and Ecology* **13**, 237–242.

Mallet, J. P., Lamouroux, N., Sagnes, P. & Persat, H. (2000). Habitat preferences of European grayling in a medium size stream, the Ain river, France. *Journal of Fish Biology* **56**, 1312–1326.

McBride, R. S., Hendricks, M. L. & Olney, J. E. (2005). Testing the validity of Cating's (1953) method for age determination of American shad using scales. *Fisheries* **30**, 10–18.

McCabe, G. D. & O'Brien, W. J. (1983). The effects of suspended silt on feeding and reproduction of Daphnia pulex. *American Midland Naturalist* **110**, 324–337.

McCormick, S. D., Hansen, L. P., Quinn, T. P. & Saunders, R. L. (1998). Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 77–92.

Metcalfe, N. B., Fraser, N. H. C. & Burns, M. D. (1999). Food availability and the nocturnal vs. diurnal foraging trade-off in juvenile salmon. *Journal of Animal Ecology* **68**, 371–381.

Michel, C., Schmidt-Posthaus, H. & Burkhardt-Holm, P. (2013): Suspended sediment pulse effects in rainbow trout (*Oncorhynchus mykiss*) - relating apical and systemic responses. *Canadian Journal of Fisheries and Aquatic Sciences* **70**, 630–641.

Mills, C. A. & Mannn, R. H. K. (1985). Environmentally-induced fluctuations in year class strength and their implications for management. *Journal of Fish Biology*, **27** (Suppl. A), 209–226.

Molls, F. (1999). New insights into the migration and habitat use by bream and white bream in the floodplain of the River Rhine. *Journal of Fish Bology* **55**, 1187–1200.

Morita, K. & Yamamoto, S. (2002). Effects of habitat fragmentation by damming on the persistence of stream-dwelling charr populations. *Conservation Biology* **16**, 1318–1323.

Muška, M., Tušer, M., Frouzová, J., Draštík, V., Čech, M., Jůza, T., Kratochvíl, M., Mrkvička, T., Peterka, J., Prchalová, M., Říha, M., Vašek, M. & Kubečka, J. (2013). To migrate, or not to migrate: partial diel horizontal migration of fish in a temperate freshwater reservoir. *Hydrobiologia* **707**, 17–28.

Nagy, S. (1984). On the age, growth and food of grayling (Thymallus thymallus L.) in the River Bela (western Tatra mountains). *Laboratorium Rybarstva a Hydrobiologie Bratislava Czechoslovakia* **4**, 301–326.

Näslund, I., Nordwall, F., Eriksson T., Hannersjö, D. & Eriksson, L.-O. (2005). Long-term responses of a stream-dwelling grayling population to restrictive fishing regulations. *Fisheries Research* **72**, 323–332.

Newcombe, C. P. & Jensen, J. O. T. (1996). Channel suspended sediment and fisheries: a synthesis for quantitative assessment of risk and impact. *North American Journal of Fisheries Management* **16**, 693–727.

Newcombe, C. P. & MacDonald, D. D. (1991). Effects of suspended sediments on aquatic ecosystems. *North American Journal of Fisheries Management* **11**, 72–82.

Northcote, T. G. (1978). Migratory strategies and production in freshwater fishes, in Ecology of Freshwater Fish Production (ed. S.D. Gerking), Blackwell, Oxford, pp. 326–359.

O´Hara, K. (1993). Fish behaviour and the management of freshwater fisheries, in Behaviour of Teleost Fishes 2nd ed. (ed. T. J. Pitcher), Chapman and Hall, London, pp. 645–670.

Oberdorff, T. & Hughes, R. (1991). Modification of an Index of Biotic Integrity based on fish assemblages to characterize rivers of the Seine Basin, France. *Hydrobiologia* **228**, 117–130.

Oberdorff, T., Pont, D., Hugueny, B. & Chessel, D. (2001). A probabilistic model characterizing fish assemblages of French rivers: a framework for environmental assessment. *Freshwater Biology* **46**, 399–415.

Oberdorff, T., Pont, D., Hugueny, B. & Porcher, J. P. (2002). Development and validation of a fish-based index (FBI) for assessment of river health in France. *Freshwater Biology* **47**, 1720–1734.

Olla, B. L., Davis, M. W. & Clifford H. R. (1998). Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. *Bulletin of Marine Science* **62**(2), 531–550.

Ovidio, M., Capra, H. & Philippart, J.-C. (2008). Regulated discharge produces substantial demographic changes on four typical fish species of a small salmonid stream. *Hydrobiologia* **609**, 59–70.

Ovidio, M., Parkinson, D. & Sonny, D. (2004). Spawning movements of European grayling *Thymallus thymallus* in the River Aisne (Belgium). *Folia Zoologica* **53**, 87–98.

Palstra, A. P. & van den Thillart, G. E. E. J. M. (2010). Swimming physiology of European silver eels (Anguilla anguilla L.): energetic costs and effects on sexual maturation and reproduction. *Fish Physiology and Biochemistry* **36**, 297–322.

Parkinson, D., Philippart, J. C. & Baras, E. (1999). A preliminary investigation of spawning migrations of grayling in a small stream as determined by radio-tracking. *Journal of Fish Biology* **55**, 172–182.

Penczak, T. (2006). Movement pattern and growth ratio of tagged fish in two lowland rivers of central Poland. *Polish Journal of Ecology* **54**, 267–282.

Persat, H. (1996). Threatened populations and conservation of the European grayling,*Thymallus thymallus* (L., 1758). In Conservation of Endangered Freshwater Fish in Europe (Kirchhofer, A. & Hefti, D., eds), pp. 233–247. Basel, Switzerland: Birkhauser Verlag.

Persat, H., Olivier J. M. & Pont, D. (1994). Theoretical habitat templets, species traits, and species richness: fish in the Upper Rhône river and its floodplain. *Freshwater Biology* **31**, 439–454.

Persson, L. (1983). Effects of intra- and interspecific competition on dynamics and size structure of a perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) population. *Oikos* **41**, 126–132.

Pienimäki, M. & Lepppäkoski, E. (2004). Invasion pressure on the Finnish Lake District: invasion corridors and barriers. *Biological Invasions* **6**, 331–346.

Pont, D., Hugueny, B., Beier, U., Goffaux, D., Melcher, A., Noble, R., Rogers, C., Roset, N. & Schmutz, S. (2006). Assessing river biotic condition at a continental scale: a European approach using functional metrics and fish assemblages. *Journal of Applied Ecology* **43**, 70–80.

Pringle, C.M. (2001). Hydrological connectivity and the management of biological reserves: a global perspective. *Ecological Applications* **11**, 981–998.

Rakowitz, G., Berger, B., Kubečka, J. & Keckeis, H. (2008). Functional role of environmental stimuli for the spawning migration in Danube nase *Chondrostoma nasus* (L.). *Ecology of Freshwater Fish* **17**, 502–514.

Redding, J. M., Schreck, C. B. & Everest, F. H. (1987). Physiological effects on coho salmon and steelhead of exposure to suspended solids. *Transactions of the American Fisheries Society* **116**, 737–744.

Reyes-Gavilan, F. G., R. Garrido, R., Nicienza, A. G., Toledo, M. M. & Brana, F. (1996). Fish community variation along physical gradients in short streams of northern Spain and the disruptive effect of dams. *Hydrobiologia* **321**, 155–163.

Rodríguez, M.A. (2002). Restricted movement in stream fish: the paradigm is incomplete, not lost. *Ecology* **83**, 1–13.

Salemaa, H. & Hietalahti, V. (1993). *Hemimysis anomala* G. O. SARS (Crustacea: Mysidacea) Immigration of a Pontocaspian mysid into the Baltic Sea. *Annales Zoologici Fennici* **30**, 271–276.

Sandström, A. & Karås, P. (2002). Effects of eutrophication on young-ofthe- year freshwater fish communities in coastal areas of the Baltic. *Environmental Biology of Fishes* **63**, 89–101.

Scheidegger, K.J. & Bain, M.B. (1995). Larval fish distribution and microhabitat use in free-flowing and regulated rivers. *Copeia* **1995(1)**, 125–135.

Schicker, K. P., Boubee, J. A. T., Stancliff, A. G. & Mitchell, C. P. (1990). Distribution of small migratory fish and shrimps in the Wailato River at Ngaruawahia, New Zealand. *Ministry of Agriculture and Fisheries*, *Freshwater Fisheries Miscellaneous Report* **63**, 21 p.

Schlosser, I. J. (1985). Flow regime, juvenile abundance, and the assemblage structure of stream fishes. *Ecology* **66**, 1484–1490.

Schmutz, S., Cowx, I. G., Haidvogl, G. & Pont, D. (2007). Fish-based methods for assessing European running waters: a synthesis. *Fisheries Management and Ecology* **14**, 369–380.

Sempeski, P. & Gaudin, P. (1995). Habitat selection by grayling-II. Preliminary results on larval and juvenile daytime habitats. *Journal of Fish Biology* **47**, 345–349.

Sempeski, P., Sagnes, P., Gaudin, P. & Statzner, B. (1997) Shifts in morphometries and their relation to hydrodynamic potential and habitat use during grayling ontogenesis. *Journal of Fish Biology* **50**, 846–858.

Sheldon, A. L. (1968). Species diversity and longitudinal succession in stream fishes. *Ecology* **49**, 193–198.

Sigler, J. W., Bjornn, T. C. & Everest, F. H., (1984). Effects of chronic turbidity on density and growth of steelheads and coho salmon. *Transactions of the American Fisheries Society* **113**, 142–150.

Slavík, O. & Bartoš, L. (2001). Spatial distribution and temporal variance of fish communities in the channelized and regulated Vltava river (Central Europe). *Environmental Biology of Fishes* **61**, 47–55.

Slavík, O. & Bartoš, L. (2004). What are the reasons for the Prussian carp expansion in the upper Elbe River, Czech Republic? *Journal of Fish Biology* **65** (Suppl. A), 240–253.

Slavík, O., Bartoš, L. & Mattas, D. (2005). Does stream morphology predict the home range size in burbot? *Environmental Biology of Fishes* **74**, 89–98.

Slavík, O., Horký, P., Bartoš, L., Kolářová, J. & Randák, T. (2007). Diurnal and seasonal behaviour of adult and juvenile European catfish as determined by radio-telemetry in the River Berounka, Czech Republic. *Journal of Fish Biology* **71**, 101–114.

Snedden, G. A., Kelso, W. E. & Rutherford, D. A. (1999). Diel and seasonal patterns of spotted gar movement and habitat use in the lower Atchafalaya river basin, Louisiana. *Transactions of the American Fisheries Society* **128**, 144–154.

Stich, H.-B. & Lampert, W. (1981). Predator evasion as an explanation of diurnal vertical migration by zooplankton. *Nature* **293**, 396–398.

Sundström, L. F. & Johnsson, J. I. (2001). Experience and social environment influence the ability of young brown trout to forage on live novel prey. *Animal Behaviour* **61**, 249–255.

Sweka, J.A. & Hartman K. J. (2003). Reduction of reaction distance and foraging success of smallmouth bass, *Micropterus dolomieu*, exposed to elevated turbidity levels. *Environmental Biology of Fishes* **67**, 341–347.

Ultsch, G. R. (1989). Ecology and physiology of hibernation and overwintering among freshwater fishes, turtles and snakes. *Biological reviews of the Cambridge Philosophical Society* **64**, 435–516.

Valdimarsson, S. K. & Metcalfe, N. B. (1998). Shelter selection in juvenile Atlantic salmon, or why do salmon seek shelter in winter? *Journal of Fish Biology* **52**, 42–49.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R. & Cushing, C. E., 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**(1), 130–137.

VanRiel, M. C., Van der Velde, G. & Bij de Vaate (2011). Dispersal of invasive species by drifting. *Current Zoology* **57**, 818–827.

Vehanen, T., Huusko, A. & Hokki, R. (2009). Competition between hatchery-raised and wild brown trout *Salmo trutta* in enclosures - do hatchery releases have negative effects on wild populations? *Ecology of Freshwater Fish* **18**, 261–268.

Vinni, M., Horppila, J., Olin, M., Ruuhijärvi, J. & Nyberg, K. (2000). The food, growth and abundance of five co-existing cyprinids in lake basins of different morphometry and water quality. *Aquatic Ecology* **34**, 421–431.

Vinyard, G.L. & O'Brien, J. (1976). Effects of light and turbidity on the reactive distance of bluegill (*Lepomis macrochirus*). *Journal of Fisheries Research Board Canada* **33**, 2845–2849.

Wheeler, A. (1969). The Fishes of the British Isles and Northwest Europe. Macmillan, London 613p.

Winter, H. V. & Fredrich, F. (2003). Migratory behaviour of ide: a comparison between the lowland rivers Elbe, Germany, and Vecht, The Netherlands. *Journal of Fish Biology* **63**, 871–880.

Witkowski, A., Kowalewski, M. & Korkurewicz, B. (1984). Grayling. Warsaw Panstwowe Wydawnictwo Rolnicze I Lesne.

Wittmann, K. J. (2007). Continued massive invasion of Mysidae in the Rhine and Danube river systems, with first records of the order Mysidacea (Crustacea: Malacostraca: Peracarida) for Switzerland. *Revue Suisse do Zoologie* **114**, 65–86.

Woolland, J. V. (1987). Grayling in the Welsh Dee Part 3, Feeding. *Journal of the Grayling Society*, Spring 15–20.

Woolland, J.V. (1986). Grayling in the Welsh Dee. *Journal of the Grayling Society*, Spring 17–20.

Woolland, J.V. (1988). The feeding relationships of grayling and trout in the Welsh Dee. *Journal of the Grayling Society*, Spring 28–29.

Wootton, R. J. (1998). Ecology of teleost fishes. Chapman & Hall, London, 404p.

CHAPTER I

Kulíšková, P., Horký, P., Slavík, O., Jones, J. I. (2009).

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Factors influencing movement behaviour and home range size in ide *Leuciscus idus*

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Seventeen individuals of ide *Leuciscus idus* were radio-tracked weekly from September 2003 to September 2004 in the River Elbe, Czech Republic, to examine migration patterns and the influence of environmental factors on their diurnal behaviour. Of the 10 environmental factors measured, *L. idus* were significantly influenced by turbidity, which increased diurnal movement and the home range size of the species. The peak of longitudinal movement occurred in the spring, indicating pre-spawning migration. Migrating fish moved downstream and later returned upstream to the vicinity of their original locations, displaying a homing behaviour.

Key words: cyprinids; insectivores; lowland river; migration; telemetry.

INTRODUCTION

It has been suggested that the daily activity rhythms of fishes are the result of a complex trade-off between growth and survival, which takes into account diel fluctuation in food availability, food capture efficiency and predation risk (Metcalfe *et al.*, 1999). Usually, such diel activity patterns are closely related to variations in the physical environment. For example, a temperature-dependent shift in diel activity is supposed to be a consequence of higher predation risk in cold water (Webb, 1978; Fraser *et al.*, 1995). Changes in diel activity patterns have been related to the 'light intensity' and duration corresponding with the daytime (Harvey & Nakamoto, 1999), 'season' (David & Closs, 2003) or the 'moon phase' (Horký *et al.*, 2006). Other environmental conditions, such as the influence of water turbidity (Benfield & Minello, 1996; Sweka & Hartman, 2003) and 'water flow' conditions, however, may have an effect (Harvey & Nakamoto, 1999; Slavík *et al.*, 2007).

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Ide *Leuciscus idus* (L.) is a benthopelagic, riverine cyprinid inhabiting deeper, slower flowing reaches of lowland middle-sized rivers east of the River Rhine basin in Europe to Siberia (Maitland & Campbell, 1992). It achieves a maximum size of 530 mm total length (L_T), a body mass of 2·0 kg and a recorded age of 14 years. *Leuciscus idus* are visually oriented feeders and predominantly consume insects, although coarse fishes or plant material may occasionally be consumed (Cala, 1970). The fish spawn in spring and belong to the phyto-lithophilic spawning group (Balon, 1975). Their migratory pattern has been described as potamodromous with a prevailing upstream migration (Cala, 1970; Müller, 1986). According to Winter & Fredrich (2003), who observed migrations of *L. idus* in the middle reaches of the River Elbe in Germany and the River Vecht in the Netherlands, the fish is a flexible species capable of adapting its movement pattern to different conditions of river systems. To investigate within catchment variation in the migratory patterns of *L. idus* the movements of individuals were tracked in a low stream order, upstream section of the River Elbe, Czech Republic, closer to the source than previous investigations (Winter & Fredrich, 2003). To assess which environmental factors influenced the migration and diurnal behaviour of the fish, 17 specimens were radio-tracked weekly from September 2003 to September 2004 in the River Elbe.

MATERIALS AND METHODS

STUDY AREA

The study was carried out on the upper part of the River Elbe. The river rises at 1383 m a.s.l. It has a total length of 1091 km with a catchment of 148 268 km^2. The Czech portion of the river is 368 km long and has a catchment of 51 394 km^2. The primary river stretch studied was *c.* 40 km long, from the weir at Střekov (distance from the source 320 km; 50°38′ N; 14°03′ E) to the frontier with Germany (Fig. 1). During spawning migrations, the stretch studied was extended as far as Meissen, Germany (distance from the source 410 km; 51°81′ N; 13°28′ E) as fish were followed. The river width in the area studied was 100–150 m, and the riverbanks have little aquatic vegetation and are reinforced with rocks and concrete. The water was up to 6 m deep and no submergent vegetation or floating plants were recorded. Across the whole study period, the average flow was 293 m^3 s^{-1}, with the maximum in winter (748 m^3 s^{-1}) and the minimum in early autumn (79 m^3 s^{-1}).

FISH CAPTURE AND TAGGING

Fish were sampled by electrofishing (650 V, 4 A and pulsed D.C.), and 17 individuals were tagged with radio transmitters. All fish were caught and released at a 5 km long river stretch (Fig. 1). The individuals were measured to the nearest millimetre (mean standard length, L_S, 378 mm ranging from 285 to 450 mm) and weighed to the nearest g (mean fish body mass 755 g and ranging from 450 to 1240 g). Fish were anaesthetized with 2-phenoxy ethanol (0·2 ml l^{-1}). Radio transmitters (MCFT 3B; 11 g in air, 14 × 43 mm, with an operational life estimated to be 399 days; MCFT 3EM; 8·9 g in air, 11 × 49 mm, with an operational life estimated to be 278 days; Lotek Engineering Inc.; www.lotek.com) were implanted into the body cavity through a midventral incision that was closed by three separate stitches, using a sterile, braided, absorbable suture (Ethicon Coated Vicryl; www.ethicon.com). The mass of the transmitter never exceeded 2% of the fish body mass in air (Winter, 1983). Fish were held until they had recovered their equilibrium and showed spontaneous swimming activity (*c.* 5 min after surgery), then released close to

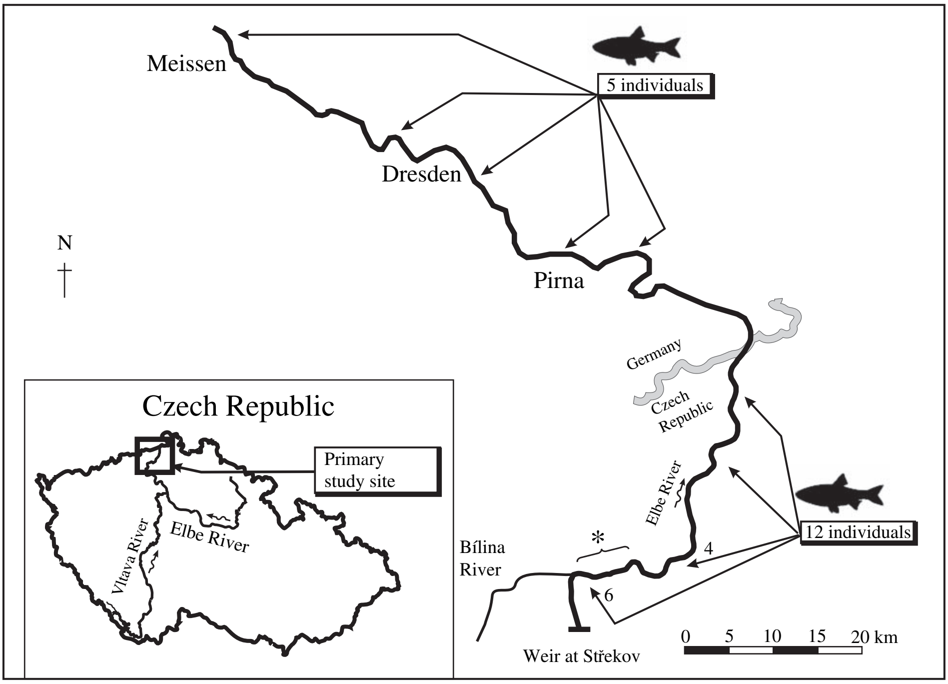

FIG. 1. Map showing the location of the study site with highlighted distances of spring downstream migrations, and number of individuals migrating within Czech and German part of River Elbe. Bracket with asterisk indicates the river stretch where the fish were caught and released after tagging. ~→, the direction of river flow.

the site of capture. The transmitters had external antennae and their potential range was *c.* 300 m depending on the gain of receiver and tracking conditions.

SAMPLING PROCEDURES

All fish were tracked from a boat weekly during the period from 11 September 2003 to 21 September 2004. Once all the fish were positioned, one individual was randomly chosen for a 24 h tracking cycle. Fish positions were determined once in each 3 h period over a diel cycle (0600–0859, 0900–1159, 1200–1459, 1500–1759, 1800–2059, 2100–2359, 2400–0259 and 0300–0559 hours) using a global positioning system (GPS) receiver. The interval between measurements varied slightly depending on the tracking conditions (180 min, range ±20 min). The fish were located using landmarks and positioned with the help of a GPS (GPS map 76S; Garmin Ltd; www.garmin.com) using two radio receivers (Lotek SRX_400 receiver firmware versions W5 and W31), and three element Yagi antennas equipped with a compass. The fish direction was determined by the double lateral extinction technique (bearing on the bisecting line of the two extinction axes; Winter *et al.*, 1978). A computer programme was developed to obtain fish position co-ordinates and plot them on the map using the biangulation method proposed by White & Garrott (1990).

HABITAT MEASUREMENT

Water temperature (°C), dissolved oxygen concentration (mg l^{-1}), conductivity (μS m^{-1}), pH and turbidity (NTU) were measured by microprocessors (Oxi 196; pH/Cond

340i/SET; TURB 355 T; WTW GmbH; www.wtw.com). Light intensity (Ev) was measured by a SEKONIC Super Zoom Master L-68 (Sekonic; www.sekonic.com) at the expected locations of individuals during each positioning. Measurements of the atmospheric pressure and the moon phase were conducted with help of the Remote Weather station BAR 928 H (Huger Electronics GmbH; www.huger.de). The Elbe River Authority measured water flow daily at a gauging station located within the study stretch.

DATA ANALYSES

Short-term movements were defined as the distance (m) between the fish positions determined in two consecutive 3 h intervals over a 24 h cycle and are henceforth referred to as 'diurnal movements'. Although the fish were located every time, in several cases the signal was so weak that a triangulation could not be precise: these occasions were excluded from further analysis. Longer-term movements were determined from the difference (m) between the locations of a fish in two successive week intervals and, henceforth, are referred to as 'longitudinal movements'. Data on fish movements were analysed using Map Source Version 5.3 (Garmin). The sizes of home ranges were determined using the minimum convex polygon method (Mohr, 1947). Fish that were used for home range analyses were suggested to occupy home range *i.e.* a fish could not move during two subsequent weeks in the longitudinal direction more than its usual extent of diurnal movements across the 24 h cycle. Furthermore, a fish that moved for the whole 24 h cycle in only one direction (upstream or downstream) was suggested to exhibit a mobile or emigration phase of a home range shift and was subsequently excluded from the analyses. Light intensity data were first entered into the analysis as the absolute values of illumination (1 Ev $\cong$ 5 lx; $y = 0{\cdot}6211e^{0{\cdot}6943x}$, where $y = \text{l}x$, $x = \text{Ev}$), referred to as 'intensity of illumination'. Furthermore, three intervals with different light intensity were determined across the 24 h cycle: twilight (light intensity ranged between 1 and 6 Ev), day (>6 Ev) and night (<1 Ev); in further analysis, these categories will be referred to as 'light intervals'.

STATISTICAL ANALYSES

Associations between the variables were tested using the linear mixed model (LMM). Separate models were applied for the following dependent variables: diurnal movements (LMM I), home range size (LMM II) and longitudinal movements (LMM III). All of the data were square-root transformed to achieve normality before analyses. To account for the repeated measurements of the same individuals across the period of observation, analyses were performed using mixed model analysis with individual fish and date nested within individual fish (LMM I, II) and individual fish and date nested within individual fish (LMM III) as a random factors, using PROC MIXED (SAS, version 9.1; SAS Institute Inc.; www.sas.com).

PROC MIXED is the way to cope with repeated measures experiments with people or animals as subjects, where subjects are declared random because they are selected from the larger population to which generalizations are wanted (SAS, 2004). For the LMM I model, fixed effect used were the classes moon phase (eight levels), season (spring, summer, autumn and winter) and light interval (day, night and twilight), and the continuous variables were turbidity (range 5·5–44 NTU), fish mass (1293–3946 g), water temperature (0–24 °C), water flow (79–748 $m^3 s^{-1}$), atmospheric pressure (992–1033 hPa), conductivity (332–425 $\mu S m^{-1}$), light intensity (0–15·1 Ev) and dissolved oxygen (5·5–12·9 $mg l^{-1}$). For the LMM II–III models, fixed effects used were the same as for the LMM I model except for the light interval (day, night and twilight) and light intensity (0–15·1 Ev) that were excluded from the analyses. The significance of each fixed effect (including interactions) in the analyses was assessed by the *F*-test, upon sequential dropping of the least significant effect, starting with a full model. Fixed effects and their interactions that were non-significant are not discussed further. In unbalanced designs with more than one effect, the arithmetic mean for a group may not accurately reflect the response for that group because other effects are unaccounted. Therefore, the least-squares means (LSMEANs) were used. LSMEANs (further referred to as 'adjusted means') are, in effect, within-group means appropriately adjusted for the other effects in the model. Adjusted means (Adj. *P*) were computed for each class; differences between classes were tested by the *t*-test. For multiple comparisons, the Tukey–Kramer adjustment was used. Associations between the dependent variable and the other continuous variables were estimated by fitting a random coefficient model using PROC MIXED as described by Tao *et al.* (2002). With this random coefficient model, predicted values for the dependent variable were calculated, and the continuous variable plotted against them with predicted regression lines. The d.f. were calculated using the Kenward–Roger method (Kenward & Roger, 1997).

RESULTS

Final LMM models contained the fixed factors turbidity for diurnal movements (LMM I), turbidity and season for home range area size (LMM II) and season for longitudinal movements (LMM III). Details of the models are shown in Table I. Descriptive data of the extent of diurnal movements, longitudinal movements, total distance migrated during spawning and home range per individual are provided in Table II. The other environmental variables tested (water temperature, dissolved oxygen, conductivity, pH, atmospheric pressure, moon phase and light intensity) were not found to have a significant effect.

DIURNAL MOVEMENTS AND HOME RANGE SIZE OF *L. IDUS*

During the whole study, fish did not remain at one exact position; however, they occupied defined home ranges between which they relocated (*e.g.* during spring migration). Mean home range size was 19 495·8 ± 13 890·9 m^2 (Table II), but both diurnal movement and home range appeared to vary in a consistent manner. Repeated measurements indicated that both diurnal movement [Fig. 2(a)] and the home range [Fig. 2(b)] of *L. idus* increased with increasing

TABLE I. Type 3 tests of fixed effects for diurnal movements, home range and longitudinal movements

Effect	Numerator d.f.	Denominator d.f.	*F*	*P*
LMM I (diurnal movement)				
Turbidity	1	256	7·82	<0·01
Light interval (season)	11	256	3·20	<0·001
LMM II (home range size)				
Turbidity	1	255	68·37	<0·001
Season	3	255	56·12	<0·001
LMM III (longitudinal movement)				
Season	3	255	5·79	<0·001

LMM, linear mixed model.

TABLE II. Tagged individuals of *Leuciscus idus* with mean ± S.D. of their recorded diurnal movements (DMs), longitudinal movements (LMs), total distance migrated during spawning (TD) and home range (HR)

Individual code	DM (m)	LM (m)	TD (m)	HR (m^2)
12	50·1 ± 100·5	1824·8 ± 5168·2	13 649	29 817·6 ± 23 924·05
14	24·8 ± 68·2	4908·7 ± 13 711·2	99 729	19 506·4 ± 14 325·2
16	39·4 ± 89·7	6372·6 ± 6803·6	13 284	23 465·8 ± 12 698·7
17	15·6 ± 24·2	135·6 ± 361·8	3882	7400·4 ± 4441·02
18	45·7 ± 95·8	5510·2 ± 10 119·3	54 160	11 342·1 ± 8432·6
24	32·8 ± 84·3	1447·8 ± 3193·4	18 633	36 157·5 ± 22 001·1
28	77·8 ± 120·1	6238·5 ± 13 801·3	85 505	24 158·2 ± 17 325·8
40	21·1 ± 54·3	1710·7 ± 1909·6	2908	8352·1 ± 5368·3
43	65·7 ± 118·6	2811·3 ± 5122·2	18 284	25 931·0 ± 18 963·2
44	34·3 ± 40·5	5517·6 ± 12 024·6	37 406	13 700·2 ± 7021·4
45	38·1 ± 81·6	982·5 ± 3421·7	4145	27 536·5 ± 19871·8
46	20·4 ± 53·8	1628·1 ± 4236·9	9380	23 658·8 ± 21 879·6
47	43·2 ± 94·4	2874·3 ± 3465·5	26 089	17 502·3 ± 13 468·2
48	37·3 ± 71·1	1382·4 ± 2678·6	4332	19 875·4 ± 13 489·7
49	19·2 ± 52·9	1348·2 ± 1825·1	3546	26 849·7 ± 21 487·2
50	28·4 ± 65·3	2949·5 ± 8236·4	67 946	13 672·3 ± 9237·4
51	30·2 ± 41·5	2131·4 ± 5628·5	13 680	2502·7 ± 2030·6

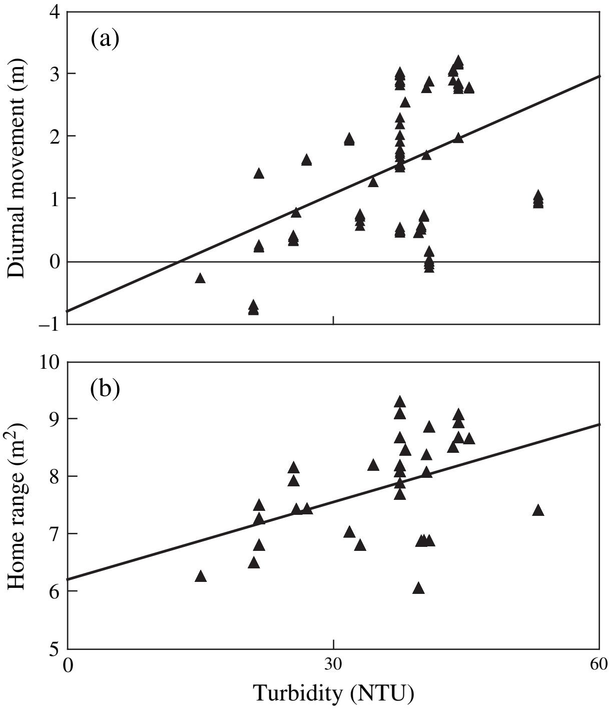

FIG. 2. The relationship between (a) diurnal movements and (b) home range of *Leuciscus idus* and turbidity. Predicted values are from square-root transformed data. The curves were fitted by: (a) $y = 0{\cdot}0188x - 0{\cdot}0395$ ($r^2 = 0{\cdot}38$) and (b) $y = 0{\cdot}0189x + 7{\cdot}4581$ ($r^2 = 0{\cdot}34$).

turbidity. The relationship between flow and turbidity was not statistically significant; increased turbidity was a consequence of both surface runoff and phytoplankton growth. Home range was significantly smaller (Tukey–Kramer Adj. $P < 0{\cdot}05$) during winter than other seasons [Fig. 3(a)].

A final LMM I model indicated the influence of the light interval nested within season on the diurnal movements of *L. idus* (Table I); however, differences among classes were insignificant, and hence the character of dependence was not possible to determine (Tukey-Kramer Adj. $P > 0{\cdot}05$).

LONGITUDINAL MOVEMENTS OF *L. IDUS*

Longitudinal movements of *L. idus* were significantly larger (Tukey-Kramer Adj. $P < 0{\cdot}01$) in the spring, with non-significant differences among other seasons [Fig. 3(b)]. Almost all individuals, with one exception, displayed downstream spring migrations, most of them remaining within Czech part of the River Elbe (40 km long; Fig. 1). Six individuals moved to near the confluence of the River Bílina at Ústí nad Labem, including one individual that moved 19 km upstream to reach this spot (the only upstream migrating individual). Four individuals moved downstream to near the town of Malé Březno and a further

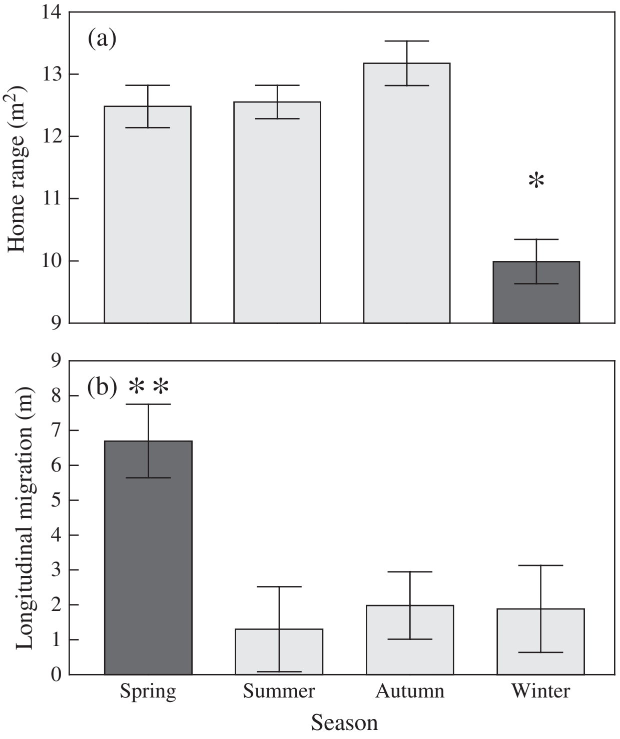

FIG. 3. (a) Home range and (b) longitudinal movements of *Leuciscus idus* across seasons. Significant differences between groups are indicated (*$P < 0{\cdot}05$; **$P < 0{\cdot}01$). Values are adjusted means ± S.E. of square-root transformed data.

two to near the border with Germany. Five individuals undertook longer migrations (68–100 km) to reach spawning sites near Dresden and Meissen in the German part of the River Elbe (Fig. 1). The individuals that undertook the longest migrations started their run earliest, at the end of February. All final destinations of migration were shallow riffles with a gravel substratum. Later in the season, *L. idus* displayed homing behaviour and returned to within 0·5–2 km of their starting position.

DISCUSSION

Behaviour of visually oriented animals is known to be affected by visibility, as they rely on visual cues for orientation and feeding. In aquatic ecosystems, the visibility is determined not only by the light intensity but also by the water turbidity (Benfield & Minello, 1996). Turbidity imposes a considerable environmental constraint with a potential to affect whole fish communities (Colby *et al.*, 1972; Diehl, 1988). It may shape the habitat choice patterns (Miner & Stein, 1996), social interactions (Valdimarsson & Metcalfe, 2001) or reproductive behaviour of the fishes, in terms of reduced sexual selection (Järvenpää & Lindström, 2004; Heubel & Schlupp, 2006). Increased turbidity influences visually oriented fishes by decreasing their visual range (Utne-Palm, 2001), typically affecting foraging efficiency by reducing the distance at which a predator detects prey (Benfield & Minello, 1996; Sweka & Hartman, 2003). Benfield & Minello (1996) evaluated the influence of turbidity on predation rates of gulf killifish *Fundulus grandis* Baird & Girard and that significantly fewer prey were consumed in tanks containing turbid water. Brook trout *Salvelinus fontinalis* (Mitchell) become more active in higher turbidity, thus increasing the chance of encountering potential prey by enlarging the total volume of water searched (Sweka & Hartman, 2001*a*). Hence, *L. idus* probably extend their diurnal movements and home range as a result of the reduced foraging success in turbid water.

In riverine systems, increased turbidity is usually associated with increased flow during hydrologic events (Sahoo *et al.*, 2006). Low discharge, however, may have the opposite effect: increased water residence time during low water flow may allow the build-up of phytoplankton biomass (Lane *et al.*, 2007). Here there was no significant relationship between discharge and turbidity, suggesting both potential sources and that the behaviour of *L. idus* was influenced by the water turbidity *per se*. Home range varied consistently with season and turbidity. The influence of turbidity is probably due to its effect on visibility. Reduced diurnal movement in winter may be due to lower food availability and temperature-related metabolism.

The winter season is a period of reduced activity in cyprinids (Bauer & Schlott, 2004). They tend to remain in areas with the most appropriate conditions for wintering, as was shown for example in bream *Abramis brama* (L.) migrating into lentic refugia (Molls & Neumann, 1994). A restricted home range may be a direct consequence of a reduced metabolic rate linked to low temperatures as well as a result of efficient energy conservation or the use of locally restricted refuge during harsh conditions (Brown & Mackay, 1995; Hiscock *et al.*, 2002).

Many freshwater fish species, including cyprinids, undertake long-distance migrations during the breeding season (Baras & Cherry, 1990; Lucas, 2000). Previous reports of *L. idus* indicated an upstream pre-spawning migration followed by downstream movement after spawning (Cala, 1970; Müller, 1986), including studies in the middle reaches of the River Elbe (Winter & Fredrich, 2003). In contrast, *L. idus* was observed to undertake similar long-distance migrations in spring but in the opposite direction, *i.e.* downstream during spring and returning upstream towards formerly occupied areas later in the season. These findings are partly consistent with Cala (1970) from Kävlingeån in Sweden, where *L. idus* also displayed large downstream migration in spring. The latter case, however, is more complicated as the fish migrated downstream to coastal waters in the spring, where they remained for the consecutive summer, only returning to the river in autumn (Cala, 1970). Such inconsistency in the direction of migration may indicate that the movements of fishes are shaped by multiple factors that vary even within river systems. An obvious constraint is the presence of lateral obstructions that hamper fish migration (Lucas & Frear, 1997; Horký *et al.*, 2007), although in the present study no fish were observed to move to the vicinity of the weir at Strekov during spring migration. The location of suitable spawning areas (Pollux *et al.*, 2006) and channel morphology (Lau *et al.*, 2006) may also be essential.

Although *L. idus* are declining in numbers, classified as vulnerable by IUCN Red List criteria (2001) and protected as an endangered species (Lusk *et al.*, 2004), few references regarding its behaviour exist (Cala, 1970; Winter & Fredrich, 2003). The present data demonstrate that the turbidity may substantially influence the movement patterns of this species. As turbidity is influenced by both eutrophication and changes in land use (Duchrow & Everhart, 1971), and increased turbidity has a negative effect on foraging success and growth of fishes (Sweka & Hartman, 2001*a*, *b*), eutrophication of the river catchment could be an important negative influence on *L. idus* abundance and distribution.

The present findings further indicate that their migratory behaviour is shaped by multiple factors that vary even within river systems. While encouragingly this may indicate a degree of plasticity in the species, more work is needed to understand the factors influencing these migrations, and hence direct conservation efforts to improve the breeding success of the remaining populations.

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References

Balon, E. K. (1975). Reproductive guilds of fishes: a proposal and definition. *Journal of Fisheries Research Board of Canada* **32,** 821–864.

Baras, E. & Cherry, B. (1990). Seasonal activities of female barbel *Barbus barbus* L. in the River Ourthe (Southern Belgium) as revealed by radio-tracking. *Aquatic Living Resources* **3,** 283–294.

Bauer, C. & Schlott, G. (2004). Overwintering of farmed common carp (*Cyprinus carpio* L.) in the ponds of a central European aquaculture facility – measurement of activity by radio telemetry. *Aquaculture* **241,** 301–317.

Benfield, M. C. & Minello, T. J. (1996). Relative effects of turbidity and light intensity on reactive distance and feeding of an estuarine fish. *Environmental Biology of Fishes* **46,** 211–216.

Brown, R. S. & Mackay, W. C. (1995). Fall and winter movements of and habitat use by cutthroat trout in Ram River, Alberta. *Transactions of the American Fisheries Society* **124,** 873–885.

Cala, P. (1970). On the ecology of the ide *Idus idus* (L.) in the River Kävlingeån, south Sweden. *Report of the Institute of Freshwater Research, Drottningholm* **50,** 45–99.

Colby, P. J., Spangler, G. R., Hurley, D. A. & McCombie, A. M. (1972). Effects of eutrophication on salmonid communities in oligotrophic lakes. *Journal of the Fisheries Research Board of Canada* **29,** 975–983.

David, B. O. & Closs, G. P. (2003). Seasonal variation in diel activity and microhabitat use of an endemic New Zealand stream-dwelling galaxiid fish. *Freshwater Biology* **48,** 1765–1781.

Diehl, S. (1988). Foraging efficiency of three freshwater fishes: effects of structural complexity and light. *Oikos* **53,** 207–214.

Duchrow, R. M. & Everhart, W. H. (1971). Turbidity measurement. *Transactions of the American Fisheries Society* **100,** 682–690.

Fraser, N. H. C., Heggenes, J., Metcalfe, N. B. & Thorpe, J. E. (1995). Low summer temperatures cause juvenile Atlantic salmon to become nocturnal. *Canadian Journal of Zoology* **73,** 446–451.

Harvey, B. C. & Nakamoto, J. R. (1999). Diel and seasonal movements by adult Sacramento pikeminnow (*Ptychocheilus grandis*) in the Eel River, north-western California. *Ecology of Freshwater Fish* **8,** 209–215.

Heubel, K. U. & Schlupp, I. (2006). Turbidity affects association behaviour in male *Poecilia latipinna*. *Journal of Fish Biology* **68,** 555–568. doi: 10.1111/j.0022-1112.2006.00941.x

Hiscock, M. J., Scruton, D. A., Brown, J. A. & Clarke, K. D. (2002). Winter movement of radio-tagged juvenile Atlantic salmon in Northeast Brook, Newfoundland. *Transactions of the American Fisheries Society* **131,** 577–581.

Horký, P., Slavík, O., Bartoš, L., Kolářová, J. & Randák, T. (2006). The effect of the moon phase and seasonality on the behaviour of pikeperch in the Elbe River. *Folia Zoologica* **55,** 411–417.

Horký, P., Slavík, O., Bartoš, L., Kolářová, J. & Randák, T. (2007). Behavioural pattern in cyprinid fish below a weir as detected by radio telemetry. *Journal of Applied Ichthyology* **23,** 679–683. doi: 10.1111/j.1439-0426.2007.00848.x

Järvenpää, M. & Lindström, K. (2004). Water turbidity by algal blooms causes mating system breakdown in a shallow-water fish, the sand goby *Pomatoschistus minutus*. *Proceedings of the Royal Society B* **271,** 2361–2365.

Kenward, M. G. & Roger, J. H. (1997). Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* **53,** 983–997.

Lane, R. R., Day, J. W. J., Marx, B. D., Reyes, E., Hyfield, E. & Day, J. N. (2007). The effects of riverine discharge on temperature, salinity, suspended sediment and chlorophyll *a* in a Mississippi delta estuary measured using a flow-through system. *Estuarine, Coastal and Shelf Science* **74,** 145–154.

Lau, J. K., Lauer, T. E. & Weinman, M. L. (2006). Impacts of channelization on stream habitats and associated fish assemblages in East Central Indiana. *American Midland Naturalist* **156,** 319–330.

Lucas, M. C. (2000). The influence of environmental factors on movements of lowland-river fish in the Yorkshire Ouse system. *The Science of the Total Environment* **251/252,** 223–232.

Lucas, M. C. & Frear, P. A. (1997). Effects of a flow-gauging weir on the migratory behaviour of adult barbel, a riverine cyprinid. *Journal of Fish Biology* **50,** 382–396.

Lusk, S., Hanel, L. & Lusková, V. (2004). Red List of the ichthyofauna of the Czech Republic: development and present status. *Folia Zoologica* **53,** 215–226.

Maitland, P. S. & Campbell, R. N. (1992). *Freshwater Fishes of the British Isles*. London: Harper Collins.

Metcalfe, N. B., Fraser, N. H. C. & Burns, M. D. (1999). Food availability and the nocturnal vs. diurnal foraging trade-off in juvenile salmon. *Journal of Animal Ecology* **68,** 371–381.

Miner, G. J. & Stein, R. A. (1996). Detection of predators and habitat choice by small bluegills: effects of turbidity and alternative prey. *Transactions of the American Fisheries Society* **125,** 97–103.

Mohr, C. O. (1947). Table of equivalent populations of North American mammals. *American Midland Naturalist* **37,** 223–249.

Molls, F. & Neumann, D. (1994). Fish abundance and fish migration in gravel-pit lakes connected with the River Rhine. *Water Science & Technology* **29,** 307–309.

Müller, K. (1986). Seasonal anadromous migration of the pike (*Esox lucius*, L.) in coastal areas of the northern Bothnian Sea. *Archiv für Hydrobiologie* **107,** 315–330.

Pollux, B. J. A., Korosi, A., Verberk, W. C. E. P., Pollux, P. M. J. & van der Velde, G. (2006). Reproduction growth, and migration of fishes in a regulated lowland tributary: potential recruitment to the River Meuse. *Hydrobiologia* **565,** 105–120. doi: 10.1007/s10750-005-1908-4

Sahoo, G. B., Ray, C. & De Carlo, E. H. (2006). Use of neural network to predict flash flood and attendant water qualities of a mountainous stream on Oahu, Hawaii. *Journal of Hydrology* **327,** 525–538.

SAS (2004). *SAS/STAT® 9.1 User's Guide*. Cary, NC: SAS Institute Inc.

Slavík, O., Horký, P., Bartoš, L., Kolářová, J. & Randák, T. (2007). Diurnal and seasonal behaviour of adult and juvenile European catfish as determined by radio-telemetry in the River Berounka, Czech Republic. *Journal of Fish Biology* **71,** 101–114. doi: 10.1111/j.1095-8649.2007.01471.x

Sweka, J. A. & Hartman, K. J. (2001*a*). Effects of turbidity on prey consumption and growth in brook trout and implications for bioenergetics modeling. *Canadian Journal of Fisheries and Aquatic Sciences* **58,** 386–393.

Sweka, J. A. & Hartman, K. J. (2001*b*). Influence of turbidity on brook trout reactive distance and foraging success. *Transactions of the American Fisheries Society* **130,** 138–146.

Sweka, J. A. & Hartman, K. J. (2003). Reduction of reactive distance and foraging success in Smallmouth bass, *Micropterus dolomieu*, exposed to elevated turbidity levels. *Environmental Biology of Fishes* **67,** 341–347.

Tao, J., Little, R., Patetta, M., Truxillo, C. & Wolfinger, R. (2002). *Mixed the SAS System Course Notes*. Cary, NC: SAS Institute Inc.

Utne-Palm, A. C. (2001). Visual feeding of fish in a turbid environment: physical and behavioural aspects. *Marine and Freshwater Behaviour and Physiology* **35,** 111–128.

Valdimarsson, S. K. & Metcalfe, N. B. (2001). Is the level of aggression and dispersion in territorial fish dependent on light intensity? *Animal Behaviour* **61,** 1143–1149.

Webb, P. W. (1978). Temperature effects on acceleration of rainbow trout, *Salmo gairdneri*. *Journal of the Fisheries Research Board of Canada* **35,** 1417–1422.

White, G. C. & Garrott, R. A. (1990). *Analysis of Wildlife Radio-tracking Data*. New York, NY: Academic Press.

Winter, H. V. & Fredrich, F. (2003). Migratory behaviour of ide: a comparison between the lowland rivers Elbe, Germany, and Vecht, The Netherlands. *Journal of Fish Biology* **63,** 871–880. doi: 10.1046/j.1095-8649.2003.00193.x

Winter, J. D. (1983). Underwater Biotelemetry. In *Fisheries Techniques* (Nielsen, L. A. & Johnsen, D., eds), pp. 371–395. Bethesda, MD: American Fisheries Society.

Winter, J. D., Kuechle, V. B., Sinff, D. B. & Tester, J. R. (1978). Equipment and methods for radio tracking freshwater fish. *University of Minnesota: Agricultural Experiment Station, Miscellaneous Reports* **152**.

CHAPTER II

Horká, P., Horký, P., Randák, T., Turek, J., Slavík, O.

Unlimited response to environmental factors: a telemetry study on the behaviour of wild and hatchery-reared European grayling *Thymallus thymallus*. *Unpublished manuscript draft.*

Unlimited response to environmental factors: a telemetry study on the behaviour of wild and hatchery-reared European grayling *Thymallus thymallus*

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Abstract

Juvenile European graylings (*Thymallus thymallus*) of wild and hatchery origin were tagged with radio-transmitters and tracked in the Blanice River in the Elbe River catchment area of the Czech Republic to study their behavioural response to stocking and environmental variability. Both wild and hatchery-reared graylings increased their diel movements and home range size according to increasing light intensity, flow, temperature and turbidity, but the characteristics of their responses differed. Wild graylings responded only up to a certain level of environmental variables, as opposed to hatchery-reared conspecifics with an un-terminated response. Hatchery-reared fish displayed larger total migration lengths over the study period (total migration) than did wild fish, which was caused mainly by their dispersal in the downstream direction.

Key words: stocking, radio-telemetry, turbidity, salmonids, migration, movement

INTRODUCTION

Stocking is routinely performed to limit the decline and/or to increase the population levels of fishes, despite the fact that it represents a significant risk for the conservation of wild indigenous populations (Persat *et al.*, 1996; Bohlin *et al.*, 2002). Artificial rearing has been shown to substantially influence the behavioural traits of fish, which may lead to reduced growth, survival rates or reproductive success of hatchery-reared fish (e.g., Olla *et al.*, 1998; Bohlin *et al.*, 2002; Weir *et al.*, 2004; Turek *et al.*, 2012). For example, hatchery-reared fish often display different foraging (Olla *et al.*, 1998; Vehanen *et al.*, 2009) or antipredator behaviours (Berejikijan *et al.*, 1999). A lack of appropriate behavioural patterns were shown to be related to previous experience (Huntingford, 1993), genetic structure (Heggberget *et al.,* 1993) or social learning (Brown & Laland, 2001).

Populations of the European grayling (*Thymallus thymallus*, L.) have substantially declined in recent decades (Persat, 1996). Accordingly, the grayling was classified as a highly vulnerable fish species according to the Bern Convention (Appendix III, Bern Convention, Council of Europe, 1979). As a typical rheophilic species, graylings were affected mostly by the loss of suitable habitats or the degradation of their quality (Mallet *et al.*, 2000), flow regulation for hydropower production (Ovidio *et al.*, 2008), overfishing (Näslund *et al.*, 2005) and migration barriers between essential habitats (Persat *et al.*, 1996). These changes imposed a considerable difficulty for this fish in achieving its entire life cycle (Mallet *et al.*, 2000; Ovidio *et al.*, 2008), and stocking was performed to support wild populations (Cowx, 1994). Recently, numerous studies have reported on post-stocking the downstream dispersal of artificially reared graylings in a natural environment (Magee & Byorth, 1994; Kaya & Jeanes, 1995; Carlstein & Ericsson, 1996; Thorve & Carlstein, 1998). Correspondingly, Turek *et al.* (2010) showed a higher site fidelity of wild graylings, which was suggested to be influenced by their experience and knowledge of the local environment.

Previous studies of grayling post-stocking behaviour were based on mark-recapture analyses. The present study attempts to determine whether the behavioural response of graylings to stocking and environmental variability would differ between individuals of wild and hatchery origin. For this purpose, juvenile graylings of wild and hatchery origin were radio-tracked in the Blanice River of the Czech Republic during the autumn-winter period for three consecutive years. The tracking period was adapted to the local management practice that is based on the stocking of two-year-old graylings during autumn.

MATERIAL AND METHODS

STUDY AREA

The study was performed on the Blanice River of the Czech Republic. The river has a total length of 93 km, with a catchment area of 860 km^2. The studied stretch was located in a fish-protected area downstream from the Husinec water reservoir at an elevation of approximately 500 m above sea level (Fig. 1). It has the characteristics of an upland river flowing through the countryside, with a broad-leaved tree forest and meadows. The river in this section consists of pools separated by riffles and stretches with slow-flowing current, and the river bed is covered by pebbles and cobbles. The river width ranges from 5 to 9 m, and the depth is 10–80 cm. The average flow was 3.5 m^3 s^{-1} for the entire study period. The dominant fish species in the studied section were brown trout (*Salmo trutta* m. *fario*, L.), European grayling (*Thymallus thymallus* L.) and stoneloach (*Barbatula barbatula* L.). Additionally, roach (*Rutilus rutilus* L.), gudgeon (*Gobio gobio* L.) and burbot (*Lota lota)*, were found occasionally.

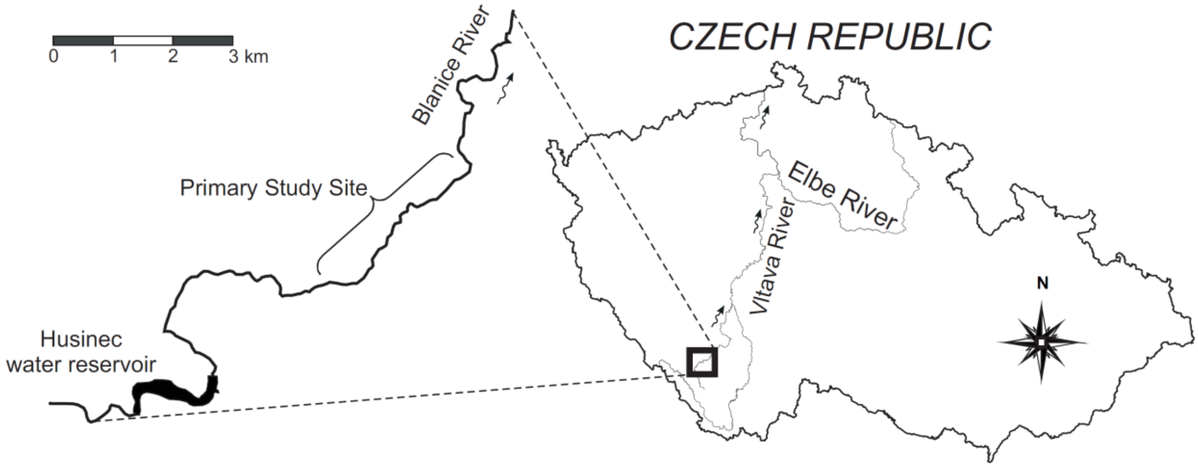

FIG. 1 Map of the study site

FISH ORIGIN AND TAGGING

A total of forty graylings (20 wild and 20 hatchery-reared fish; mean standard length [L_S] 195 mm, range of 139–239 mm; mean weight of 99 g, range of 68–150 g) were marked for the radio-telemetry study. The fish were anaesthetised with 2-phenoxy ethanol (0.2 ml l^{-1}), and radio transmitters (NTC-3-2 KMF; 1.2 g in air, 7.3×18 mm, with an estimated operational life of 80 days; Lotek Engineering Inc.; www.lotek.com) were implanted into the body cavity through a midventral incision that was closed with three separate stitches using a sterile, braided, absorbable suture (Ethicon Coated Vicryl; www.ethicon.com). The mass of the transmitter never exceeded 2% of the body mass in air (mean tag ratio 1.21%, range 0.8–1.76%; Winter, 1983). The fish were held until they had recovered their equilibrium and showed spontaneous swimming activity (ca. 5 min after surgery) and were then released into the river. The hatchery-reared fish were the progeny of resident wild broodstock in the Blanice River. They originated from artificial spawning and were reared from the fingerling stage in concrete tanks. They were fed on conventional dry food pellets and were held in the Husinec Hatchery (Czech Anglers' Union), supplied with water from the Blanice River. The rearing facilities were situated ca. 5–7 km upstream from the study area. Wild fish of corresponding size originated from the natural spawning and were caught by electrofishing (FEG 1500, EFKO-Germany; www.efkogmbh.de, pulsed D.C.).

SAMPLING PROCEDURES

Twenty fish (10 wild and 10 hatchery-reared fish) were tracked weekly during two separate tracking periods (November 2007 - January 2008 and November 2008 - January 2009) for 12 consecutive weeks. Once the position of all of the fish was determined, several individuals (5 on average) were randomly chosen for a 24 h tracking cycle. Whenever possible, attention was paid to keep the number of hatchery-reared and wild fish for a particular 24 h tracking cycle balanced. Fish positions were determined once in each 3 h period over a diel cycle (0600–0859, 0900–1159, 1200–1459, 1500–1759, 1800–2059, 2100–2359, 2400–0259 and 0300–0559 hours). The fish were located using landmarks and positioned utilising a GPS (GPS map 76S; Garmin Ltd; www.garmin.com) with two radio receivers (Lotek SRX_400 receiver firmware versions W5 and W31) and three-element Yagi antennas equipped with a compass. The fish direction was determined by the double lateral extinction technique (bearing on the bisecting line of the two extinction axes; Winter *et al.*, 1978). A computer programme was developed to obtain fish position co-ordinates and plot them on the map using the biangulation method proposed by White & Garrott (1990).

HABITAT MEASUREMENTS

The water temperature (°C), dissolved oxygen (mg L^{-1}), pH, conductivity (µS cm^{-1}) and turbidity (NTU) were measured using corresponding field probes (Oxi 196 WTW; pH/Cond 340i SET; TURB 355 T; WTW GmbH; www.wtw.com). The light intensity (Ev) was measured by a SEKONIC Super Zoom Master L-68 (Sekonic; www.sekonic.com). All of these measurements were performed throughout the study during the days when the fish were tracked. The flow (m^3 s^{-1}) was automatically recorded daily at the gauging station located within the study stretch.

DATA ANALYSES

Data from 38 graylings were included in our statistical analyses. Two hatchery-reared individuals were lost for unknown reasons after release and thus were excluded from further analyses. Short-term movements were defined as the distance (m) between the fish positions determined in two consecutive 3 h intervals over a 24 h cycle and are henceforth referred to as ‘diel movements’. Longer-term movements were determined from the difference (m) between the locations of a fish in two successive week intervals and, henceforth, are referred to as ‘longitudinal movements’. The ‘total migration’ of a specimen was computed as the distance between the two farthest positions of a fish during the entire study period. The fish movement data were analysed using Map Source Version 5.3 (Garmin).

The ‘home range size’ was determined using the minimum convex polygon method (Mohr, 1947). Fish that were used for the home range analyses were considered to occupy the home range, i.e., when a fish did not move during two subsequent weeks in the longitudinal direction more than its usual extent of diel movements across the 24 h cycle. A fish that moved for the whole 24 h cycle in only one direction (upstream or downstream) was considered to exhibit a mobile or emigration phase of a home range shift and was subsequently excluded from the analyses. To ensure that diurnal and longitudinal movements, total migration and home range were independent of fish length LS, these variables were corrected by dividing by the individual fish LS (Aarestrup *et al.*, 2005). In further analyses, we used values correcting for fish length only. Light intensity data were first entered into the analysis as the absolute values of illumination (1 Ev), referred to as the ‘intensity of illumination’. Furthermore, three intervals with different light intensities were determined across the 24 h cycle: twilight (light intensity ranging from between 1 and 6 Ev), day (>6 Ev) and night (<1 Ev); in further analyses, these categories will be referred to as ‘light intervals’.

STATISTICAL ANALYSIS

The statistical analyses were performed using the SAS software package (SAS Institute Inc., version 9.2, www.sas.com). The data were transformed to meet normality requirements when needed. The grayling diel movements, home range size, longitudinal movements and total migration were analysed using a linear mixed model (LMM) with random factors (PROC MIXED). The random factors were used to account for repeated measures collected for the same experimental units (individual fish) across the duration of the experiment. The significance of each exploratory variable (i.e., fixed effect, including their interactions) in the particular model was assessed using an F-test in which we sequentially dropped the least significant effect, beginning with the full model (backward selection procedure). Least-squares means (LSM), henceforth referred to as ‘adjusted means’, were computed for each significant class exploratory variable. Differences between the classes were tested with a t-test. We used a Tukey–Kramer adjustment for multiple comparisons. Associations between the dependent variables and other continuous variables were estimated by fitting a random coefficient model using PROC MIXED as described by Tao *et al.* (2002). With this random coefficient model, we calculated the predicted values for the dependent variables and plotted them against the continuous variables by using the predicted regression lines. The degrees of freedom were calculated using the Kenward-Roger method (Kenward & Roger, 1997).

The data on grayling diel upstream/downstream movements were subjected to a $\chi 2$ test. The possible detailed differences between the movements of wild and hatchery-reared fish were analysed using the generalised estimating equation (GEE) approach (Liang & Zeger, 1986) of categorical, repeated measurements using the GENMOD procedure with binomial distributions. GEE is an extension of generalised linear models that provides a semi-parametric approach to longitudinal data analysis. In this study, the GENMOD procedure was applied to estimate the probability of moving upstream or downstream during the diel movements across the 24 h cycle.

RESULTS

In total, we analysed 928 records of grayling positions. The observed behavioural variables varied in the following manner: the diel movements ranged from 0 to 98 m; home range size from 21 to 1428 m^2; longitudinal movements from 0 to 4125 m and total migration from 31 to 4,367 m.

Wild and hatchery-reared fish responded to the same environmental factors, but the characteristics of these relationships depended upon the origin of the fish. In general, the response of wild fish to environmental variability had a threshold-related character. Wild fish usually responded to stimuli only up to/from a certain level, and the effect of a further increase/decrease of the measured variable on their behaviour levelled out, whereas hatchery-reared fish responded with continuous increases in the intensity of the observed behaviour. This difference was observed for the diel movements that increased with increasing light intensity and turbidity levels ($F_{2,880}$=4.85, P<0.01; Fig. 2 a, b); home range size that increased with turbidity and flow ($F_{2,860}$=5.08, P<0.01; Fig. 3 a, b) as well as flow and temperature ($F_{2,860}$=5.72, P<0.01; Fig. 4 a, b) and longitudinal movements that increased with flow and temperature ($F_{2,220}$=4.51, P<0.01; Fig. 5 a, b).

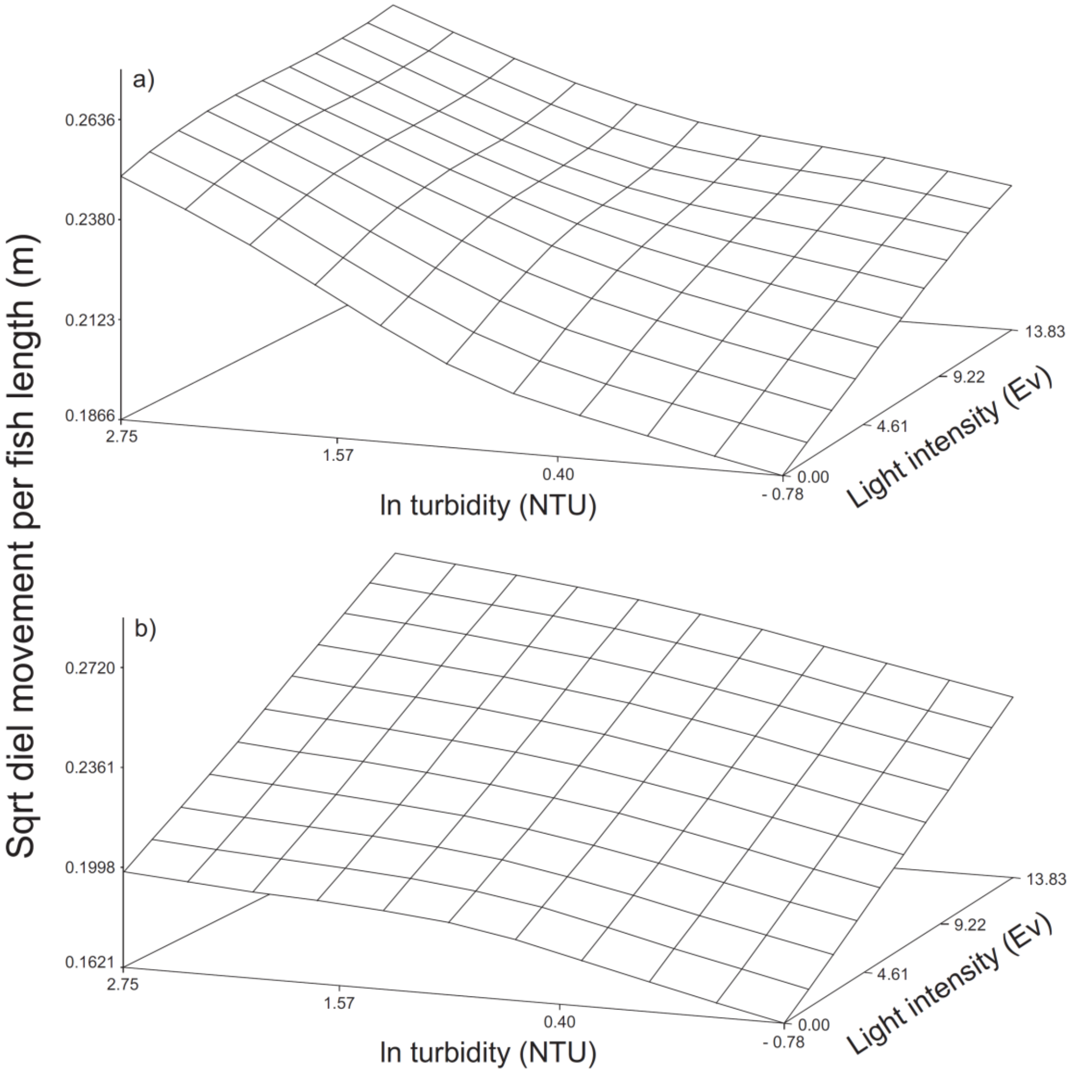

FIG. 2 The relationships between diel movement per fish length, turbidity and light intensity for hatchery-reared (a) and wild (b) European graylings (*Thymallus thymallus)*.

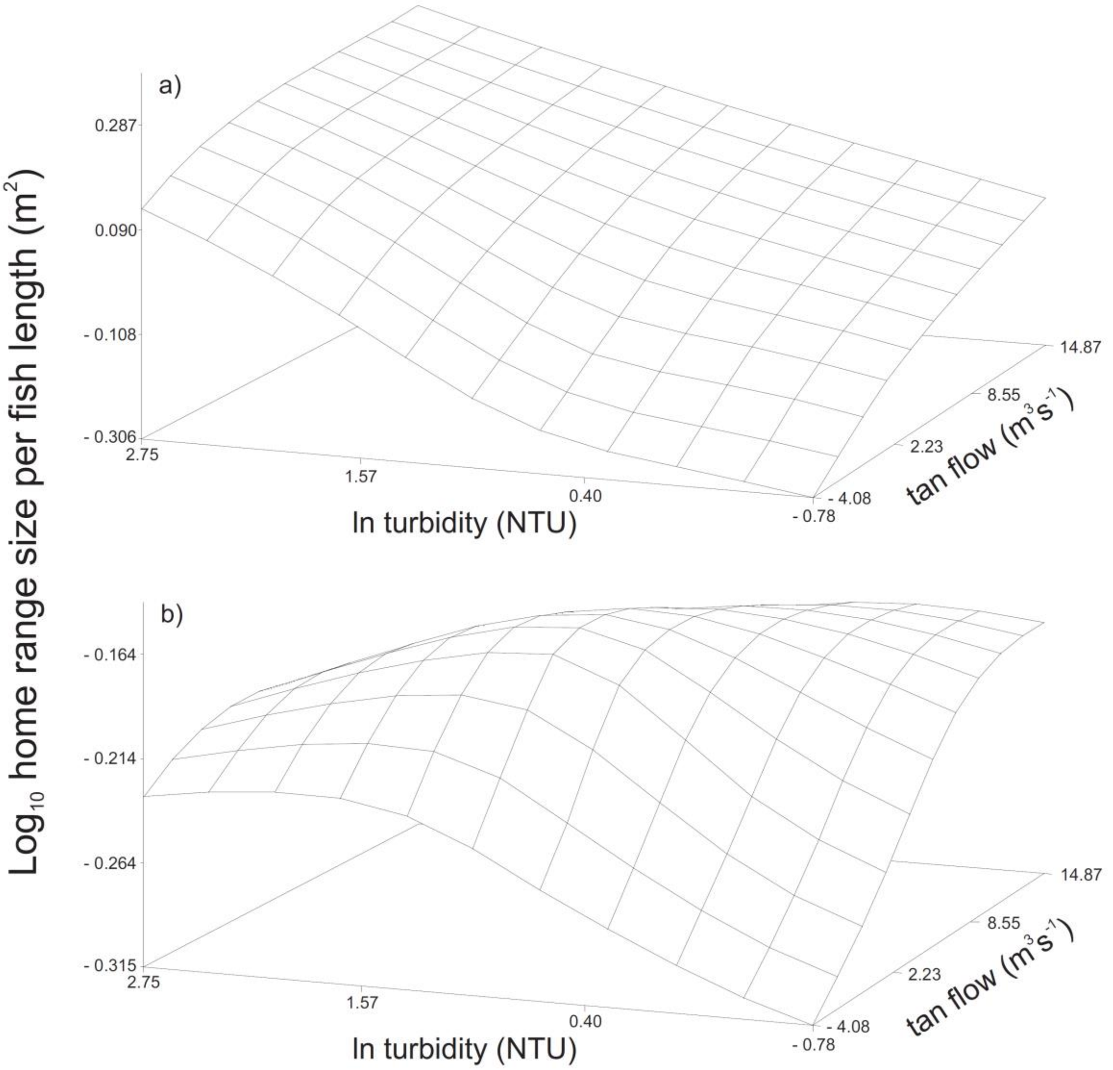

FIG. 3 The relationship between home range per fish length, turbidity and flow for hatchery-reared (a) and wild (b) European graylings (*Thymallus thymallus).*

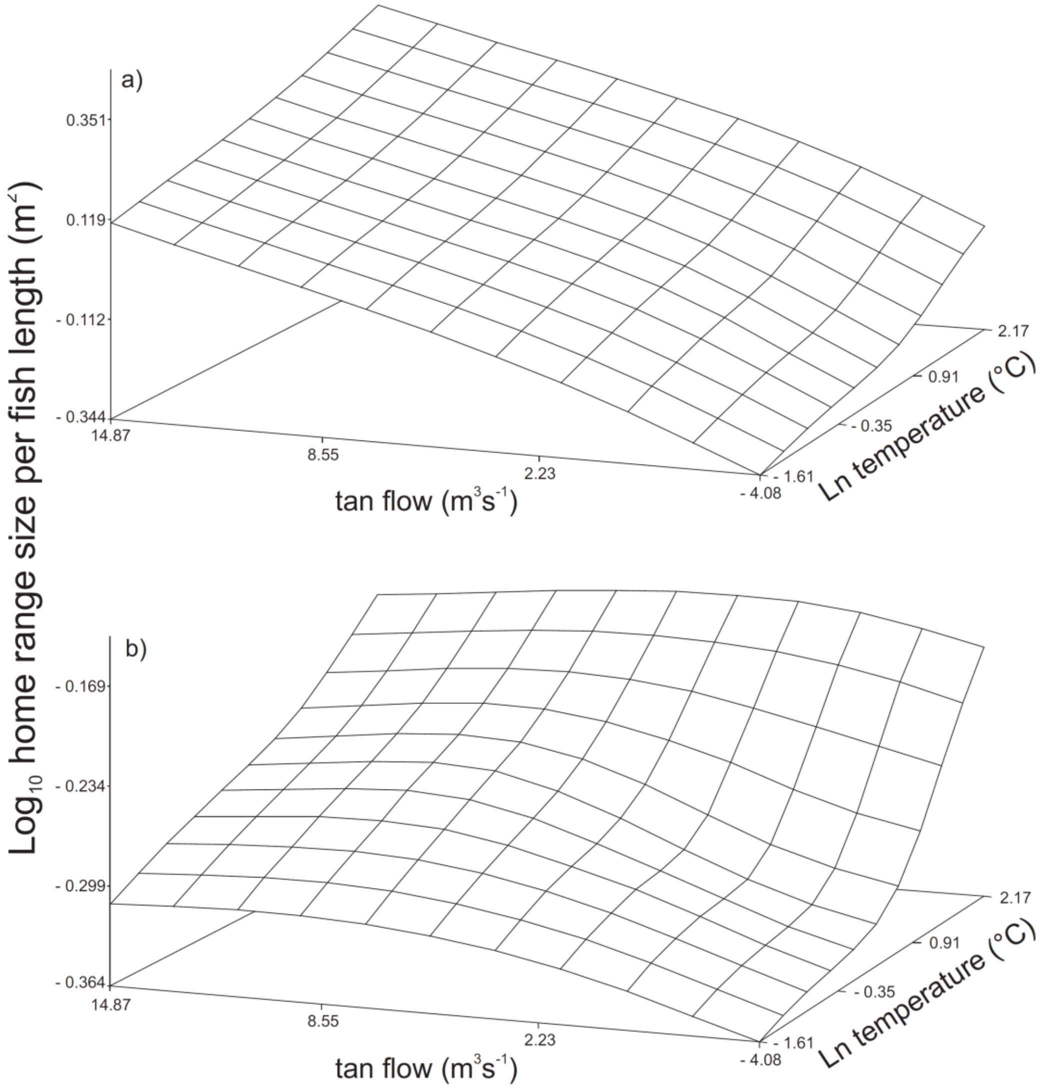

FIG. 4 The relationship between home range per fish length, flow and temperature for hatchery-reared (a) and wild (b) European graylings (*Thymallus thymallus).*

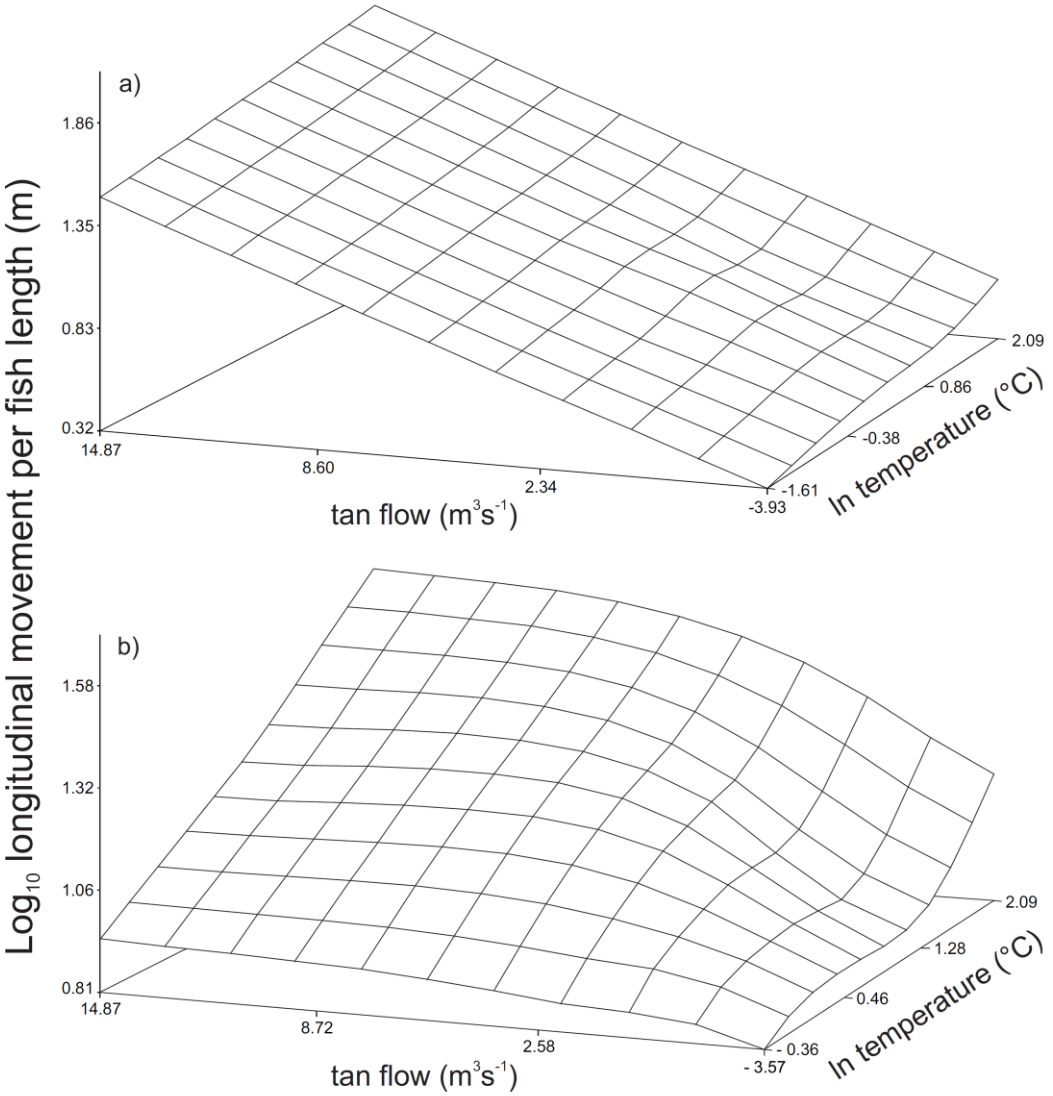

FIG. 5 The relationship between longitudinal movement per fish length, flow and temperature for hatchery-reared (a) and wild (b) European graylings (*Thymallus thymallus).*

The diel movements of hatchery-reared fish were lower during the night ($F_{4,778}$=6.95, P<0.0001; Fig. 6), whereas the difference in wild fish diel activity across time intervals was non-significant. The probability of moving upstream during the diel cycle was higher for wild than for hatchery-reared fish (χ2=6.53, d.f.=1; P<0.01; Fig. 7), while there was no difference in the probability of displaying downstream diel movements.

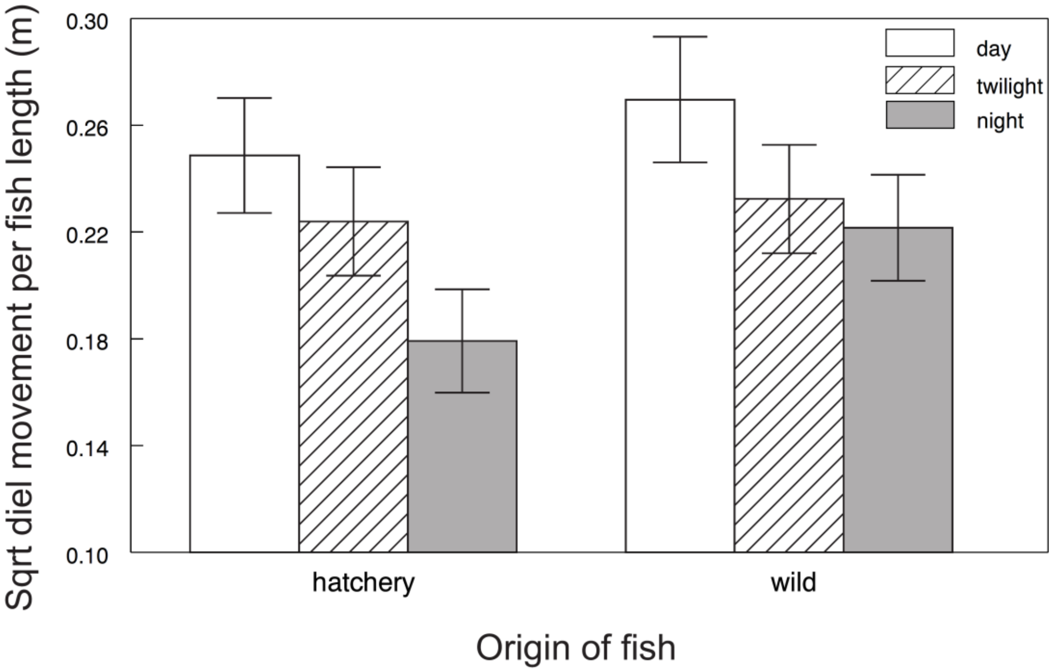

Fig 6 The relationship between diel movement per fish length and origin of fish across time intervals.

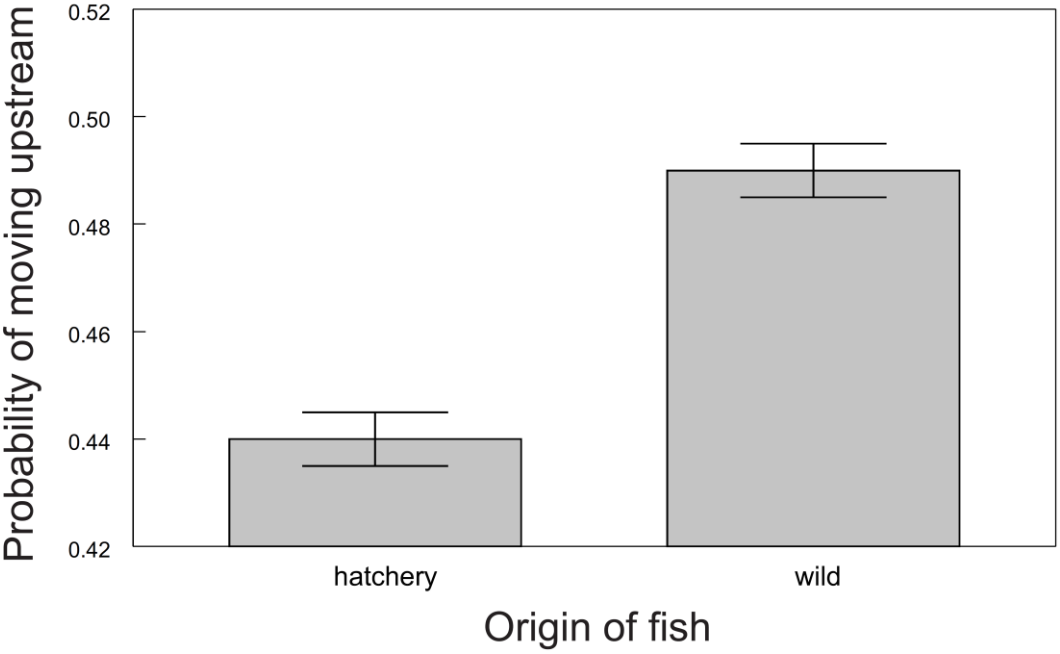

FIG. 7 Probability of moving upstream during diel movements according to the origin of the fish.

Hatchery-reared fish displayed a larger total migration than did wild fish ($F_{1,38}$=5.75, P<0.05; Fig. 8). This difference was caused mainly by their downstream dispersal from the point of release ($F_{1,38}$=3.83, P<0.05), as the upstream dispersal was not significantly influenced by the origin of the fish ($F_{1,38}$=1.63, P<0.2).

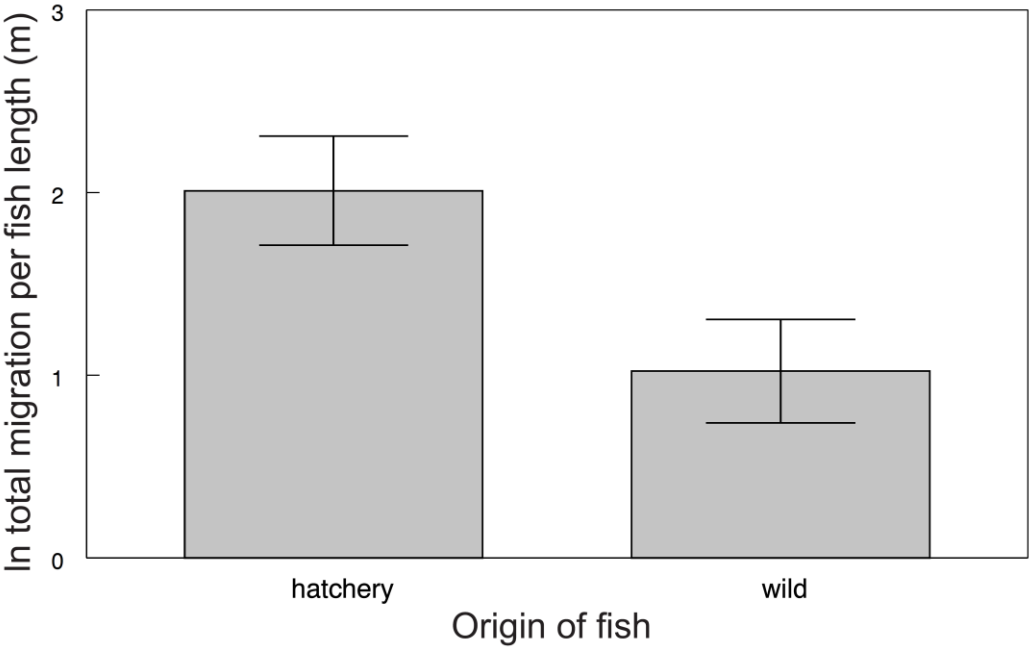

FIG. 8 Total migration per fish length according to the origin of the fish.

DISCUSSION

The main difference between hatchery-reared and wild graylings was in the characteristics of their behavioural responses to environmental factors. Wild fish usually responded to stimuli only up to/from a certain level. The effect of further changes of the measured variable on their behaviour levelled out, suggesting a threshold-related dependence. In contrast, hatchery-reared fish responded with continuous increases in the intensity of the observed behaviour. As the hatchery-reared graylings grew up in a different environment (Olla *et al.*, 1998), with no experience of natural river conditions (Jensen *et al.*, 1986) or the opportunity to learn favourable abilities (Vehanen *et al.*, 2009), they likely did not develop such adaptive responses as their wild conspecifics. Behavioural deficiencies of hatchery-reared fish in a natural environment were shown in many aspects of feeding, antipredator or reproductive behaviour (see Huntingford *et al.*, 2004 for review). Nevertheless, poorer adaptations to the local environmental conditions could also be a result of differences in genetics between wild and hatchery fish (Heggberget, 1993).

The increased movement of graylings in turbid water may be viewed as a result of a reduced level of perceived predation risk or foraging success (Abrahams & Kattenfeld, 1997; Kulíšková *et al.*, 2009). Turbidity as protection from predators is of importance, as was shown with the reduced frequency of refuge use by rainbow trout (*Oncorhynchus mykiss)* (Walbaum) in turbid water (Gregory & Griffith, 1996). According to Sweka & Hartman (2001), brook trout (*Salvelinus fontinalis)* (Mitchell) become more active in higher turbidity, thus increasing the chance of encountering potential prey by enlarging the total volume of the water searched. However, with a further increase in turbidity, the fish response may change again: Gregory & Northcote (1993) found that the feeding rates for surface and benthic prey in juvenile chinook salmon (*Oncorhynchus tshawytscha)* (Walbaum) were the highest at intermediate turbidity levels. Although we were not able to quantify feeding rates, the behavioural response of the wild graylings was analogous and may suggest their experience with balancing movement-feeding efficiency trade-offs in different turbidity levels. In contrast, the un-terminated behavioural response of the hatchery-reared graylings could be viewed as a result of a lack of juvenile experience from the river (Jonsson *et al.,* 1990).

The water flow and temperature have often been considered to be the major environmental factors influencing fish movement and home range size (Slavík & Bartoš, 2002; Nykänen *et al.*, 2004; Riley *et al.*, 2009), and indeed these factors also affected the studied graylings. Temperature has a strong impact on many aspects of fish biology, particularly with regard to positively temperature-dependent metabolic rates (Brett, 1964). The maximum swimming speed, important in escape from a predator or attacking prey, is also influenced by temperature (Webb, 1978). The enlargement of the grayling home range and longitudinal movements during high flows could either result from an increase in food availability (Tesch, 1977; LaBar *et al.*, 1987) or the tendency to occupy the most profitable energetic positions (Harvey & Nakamoto, 1999).

The diel movements of juvenile graylings increased with the light intensity. Nevertheless, hatchery-reared fish displayed a significantly lower night time activity, while wild fish did not. The more intense nocturnal movement of wild fish may be connected with feeding and/or predator avoidance (Vanderpham *et al.*, 2012); this difference was noted for related species, such as for brown trout (Diana *et al.*, 2004) or Atlantic salmon (Fraser *et al.*, 1993). The night time activity of wild graylings could also be related to seasonal changes in behaviour; the graylings in our study were observed during the autumn-winter period, which is often reported to be associated with night time activity in salmonids (Fraser *et al.*, 1993; Valdimarsson & Metcalfe, 1998). Reduced nocturnal activity in hatchery-reared graylings may be caused by the lack of experience with the river environment. A similar phenomenon was described by Lucas (2000): most of the diel activity in wild riverine cyprinids, mainly chub and dace, occurred during the dusk/night-time period, while hatchery-reared fish exhibited mainly daytime activity.

In our study, hatchery-reared graylings displayed a larger total migration, particularly in the downstream direction. The post-stocking downstream dispersal of hatchery-reared graylings corresponds with previous findings (e.g., Carlstein & Eriksson, 1996). Many studies of salmonids have shown that the behaviour of hatchery-reared fishes differs from that of wild fish, mainly in the degree of aggressiveness or feeding behaviour (Deverill *et al.*, 1999; Sundström & Johnsson 2001; Huntingford 2004). Such studies often suggest that fish of wild origin have a prior-resident competitive advantage over later-introduced hatchery-reared individuals (Deverill *et al.*, 1999). The general grayling response to danger of escaping from the area (Thorfve & Carlstein, 1998) may also be of importance in their downstream dispersal after stocking into a novel environment. Based on these findings, it can be assumed that the larger total migration of hatchery-reared graylings in our study is most likely a consequence of progressive adaptation to the local environment and competition for feeding and/or sheltering sites with the residents.

Overall, our study supports the view that fishes adapt during ontogenesis in hatchery to strikingly different environments, which shape their behaviour in many respects (Huntingford, 2004). The influence of the hatchery environment on grayling behavioural deficits in the wild is obvious; therefore, habitat complexity and natural spawning renewal should be of the first importance instead of stocking in attempts to conserve indigenous grayling populations.

Acknowledgements

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References

Aarestrup, K., Jepsen, N., Koed, A. & Pedersen. S. (2005). Movement and mortality of stocked brown trout in a stream. *Journal of Fish Biology* **66**, 721–728.

Abrahams, M. & Kattenfeld, M. (1997). The role of turbidity as a constraint on predator-prey interactions in aquatic environments. *Behavioral Ecology and Sociobiology* **40**, 169–174.

Berejikian, B. A., Smith, R. J. F., Tezak, E. P., Schroder, S. L. & Knudsen, C. M. (1999). Chemical alarm signals and complex hatchery rearing habitats affect antipredator behavior and survival of chinook salmon (*Oncorhynchus tshawytscha*) juveniles. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 830–838.

Bern Convention (1979). Convention on the conservation of European wildlife and natural habitats, Bern/Berne, Council of Europe, 19/09/1974.

Bohlin, T., Sundström, L. F., Johnsson, J. I., Höjesjö, J. & Pettersson, J. (2002). Density-dependent growth in brown trout: effects of introducing wild and hatchery fish. *Journal of Animal Ecology* **71**, 683–692.

Brett, J. R. (1964). The respiratory metabolism and swimming performance of young sockey salmon. *Journal of Fisheries Research Board Canada* **21**, 1183–1226.

Brown, C. & K. Laland (2001). Social learning and life skills training for hatchery reared fish. *Journal of Fish Biology* **59**, 471–493.

Carlstein, M. & Eriksson, L.-O. (1996). Post-stocking dispersal of European grayling, *Thymallus thymallus* (L.), in a semi-natural experimental stream. *Fisheries Management and Ecology* **3**, 143–155.

Cowx, I. G. (1994). Stocking strategies. *Fisheries Management and Ecology* **1**, 15–30.

Deverill, J. I., Adams, C. E. & Bean, C. W. (1999). Prior residence, aggression and territory acquisition in hatchery-reared and wild brown trout. *Journal of Fish Biology* **55**, 868–875.

Diana, J. S., Hudson, J. P. & Clark, R. D. (2004). Movement patterns of large brown trout in the mainstream Au Sable River, Michigan. *Transactions of the American Fisheries Society* **133**, 33–44.

Fraser, N. H. C., Metcalfe, N. B. & Thorpe, J. E. (1993). Temperature-dependent switch between diurnal and nocturnal foraging in salmon. *Proceedings of the Royal Society London B* **252**, 135–139.

Gregory, J. S. & Griffith, J. S. (1996). Winter concealment by subyearling rainbow trout: space size selection and reduced concealment under surface ice and in turbid water conditions. *Canadian Journal of Zoology* **74**, 451–455.

Gregory, R. S. & Northcote, T. G. (1993). Surface, planktonic, and benthic foraging by juvenile chinook salmon (*Oncorhynchus tschawytscha*) in turbid laboratory conditions. *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 233–240.

Harvey, B. C. & Nakamoto, J. R. (1999). Diel and seasonal movements by adult Sacramento pikeminnow (*Ptylocheilus grandis*) in the Eel River, north-western California**.** *Ecology of Freshwater Fish* **8**, 209–215.

Heggberget, T. G., Johnsen, B. O., Hindar, K., Jonsson, B., Hansen, L. P., Hvidsten, N. A. & Jensen, A. J. (1993). Interactions between wild and cultured Atlantic salmon: a review of the Norwegian experience. *Fisheries Research* **18**, 123–146.

Huntingford, F. A. (1993). Development of behaviour in fish. In: Behaviour of Teleost Fishes (Ed. by T. J. Pitcher), pp. 57–83. London: Chapman and Hall.

Huntingford, F. A. (2004). Implications of domestication and rearing conditions for the behaviour of cultivated fishes. *Journal of Fish Biology* **65** (Supplement A), 122–142.

Jensen, A. J., Heggberget, T. G. & Johnsen, B. O. (1986). Upstream migration of adult Atlantic salmon, *Salmo salar* L., in the River Vefsna, northern Norway. *Journal of Fish Biology* **26**, 459–465.

Jonsson, B., Jonsson, N. & Hansen, L. P. (1990). Does juvenile experience affect migration and spawning of adult Atlantic salmon? *Behavioral Ecology and Sociobiology* **26**, 225–230.

Kaya, C. M. & Jeanes, E. D. (1995). Retention of adaptive rheotactic behaviour by F1 fluvial Arctic grayling. *Transactions of the American Fisheries Society* **124,** 453–457.

Kenward, M. G. & Roger, J. H. (1997). Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* **53**, 983–997.

Kulíšková, P., Horký, P., Slavík, O., & Jones, J. I. (2009). Factors influencing movement behaviour and home range size in ide *Leuciscus idus*. *Journal of Fish Biology* **74**, 1269–1279.

LaBar, G. W., Hernando Casal, J. A. & Delgado, C. F. (1987). Local movements and population size of European eels, *Anguilla anguilla*, in a small lake in south western Spain. *Environmental Biology of Fishes* **19**, 111–117.

Liang, K. Y. & Zeger, S. L. (1986). Longitudinal data analysis using generalized linear models. *Biometrika* **73**, 13–22.

Lucas, M. C. (2000). The influence of environmental factors on movements of lowland-river fish in the Yorkshire Ouse system. *The Science of the Total Environment* **251/252**, 223–232.

Magee, J. P. & Byorth, P. A. (1994). Competitive Interactions of Fluvial Arctic Grayling (*Thymallus arcticus*) and Brook Trout (*Salvelinus fontinalis*) in the Upper Big Hole River, Montana. Dillon, MT: Montana Department of Fish, Wildlife and Parks.

Mallet, J. P., Lamouroux, N., Sagnes, P. & Persat, H. (2000). Habitat preferences of European grayling in a medium size stream, the Ain river, France. *Journal of Fish Biology* **56**, 1312–1326.

Mohr, C. O. (1947). Table of equivalent populations of North American mammals. *American Midland Naturalist* **37**, 223–249.

Näslund, I., Nordwall, F., Eriksson T., Hannersjö, D. & Eriksson L.-O. (2005). Long-term responses of a stream-dwelling grayling population to restrictive fishing regulations. *Fisheries Research* **72**, 323–332.

Nykänen, M., Huusko, A. & Lahti, M. (2004). Changes in movement, range and habitat preferences of adult grayling from late summer to early winter. *Journal of Fish Biology* **64**, 1386–1398.

Olla, B. L., Davis, M. W. & Clifford H. R. (1998). Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. *Bulletin of Marine Science* **62**(2), 531–550.

Ovidio, M., Capra, H. & Philippart, J.-C. (2008). Regulated discharge produces substantial demographic changes on four typical fish species of a small salmonid stream. *Hydrobiologia* **609**, 59–70.

Persat, H. (1996). Threatened populations and conservation of the European grayling, *Thymallus thymallus* (L., 1758). In Conservation of Endangered Freshwater Fish in Europe (Kirchhofer, A. & Hefti, D., eds), pp. 233–247. Basel, Switzerland: Birkhauser Verlag.

Riley, W. D., Maxwell, D. L., Pawson, M. G. & Ives, M. J. (2009). The effects of low summer flow on wild salmon (*Salmo salar*), trout (*Salmo trutta*) and grayling (*Thymallus thymallus*) in a small stream. *Freshwater Biology* **54**, 2581–2599.

Slavík, O. & Bartoš, L. (2002). Factors affecting migrations of burbot. *Journal of Fish Biology* **60**, 989–998.

Sundström, L. F. & Johnsson, J. I. (2001). Experience and social environment influence the ability of young brown trout to forage on live novel prey. *Animal Behaviour* **61**, 249–255.

Sweka, J. A. & Hartman, K. J. (2001). Effects of turbidity on prey consumption and growth in brook trout and implications for bioenergetics modeling. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 386–393.

Tao, J., Little, R., Patetta, M., Truxillo, C. & Wolinger R. (2002). Mixed the SAS System Course Notes. Cary, NC: SAS Institute Inc.

Tesch, F. W. (1977). The Eel – Biology and Management of Anguillid Eels. London: Chapman & Hall.

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**, 147–159.

Turek, J., Horký, P., Žlábek, V., Velíšek, J., Slavík, O. & Randák, T. (2012). Recapture and condition of pond-reared and hatchery-reared 1+ European grayling stocked in additon to wild conspecifics in a small river. *Knowledge and Management of Aquatic Ecosystems* **405**, 10.

Turek, J., Randák, T., Horký, P., Žlábek, V., Velíšek, J., Slavík, O. & Hanák, R. (2010). Post-release growth and dispersal of pond and hatchery-reared European grayling *Thymallus thymallus* compared with their wild conspecifics in a small stream. *Journal of Fish Biology* **76**, 684–693.

Valdimarsson, S. K. & Metcalfe, N. B. (1998). Shelter selection in juvenile Atlantic salmon, or why do salmon seek shelter in winter? *Journal of Fish Biology* **52**, 42–49.

Vanderpham, J. P., Nakagawa, S. & Closs, G. P. (2012). Diel variation in use of cover and feeding activity of a benthic freshwater fish in response to olfactory cues of a diurnal predator. *Environmental Biology of Fishes* **93**, 547–556.

Vehanen, T., Huusko, A. & Hokki, R. (2009). Competition between hatchery-raised and wild brown trout *Salmo trutta* in enclosures - do hatchery releases have negative effects on wild populations? *Ecology of Freshwater Fish* **18**, 261–268.

Webb, P. W. (1978). Temperature effects on acceleration of rainbow trout, *Salmo gairdneri*. *Journal of Fisheries Research Board Canada* **35**, 1417–1422.

Weir, L. K., Huthings, J. A., Fleming, I. A. & Einum, S. (2004). Dominance relationships and behavioural correlates of individual spawning success in farmed and wild male Atlantic salmon, *Salmo salar*. *Journal of Animal Ecology* **73**, 1069–1079.

White, G. C. & Garrott, R. A. (1990). Analysis of wildlife radio-tracking data. New York,NY: Academic Press.

Winter, J. D. (1983). Underwater Biotelemetry. In *Fisheries Techniques* p. 371-395. (Nielsen, L. A. & Johnsen, D., eds), pp. 371–395. Bethesda, MD: American Fisheries Society.

Winter, J.D., Kuechle, V.B., Sinff, D.B. & Tester, J.R. 1978. Equipment and methods for radio tracking freshwater fish. Univ. Minessota: Agricultural Experiment Station, Miscellaneous Reports 152.

CHAPTER III

Horká, P., Horký, P., Slavík, O., Opatřilová, L. (2012).

Diurnal behavioural patterns and spread of the Ponto-Caspian invader *Hemimysis anomala* G. O. SARS, 1907 (Crustacea, Mysidacea) in the Elbe River, Czech Republic. *International Review of Hydrobiology* 97, 454–462.

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Research Paper

Diurnal behavioural patterns and spread of the Ponto-Caspian invader *Hemimysis anomala* G. O. SARS, 1907 (Crustacea, Mysidacea) in the Elbe River, Czech Republic

key words: biological invasions, non-indigenous species, mysid shrimp, migration, turbidity

Abstract

The Ponto-Caspian invader *Hemimysis anomala* was studied in the river Elbe, Czech Republic. The study confirmed further spread of the species: the presence of juveniles and gravid females indicated that *H. anomala* is established within the Elbe River. Bottom, mid-water and surface drift samples were collected during 24-h cycles from July to September 2007. A tendency to remain hidden was the main behavioural strategy of *H. anomala* in the riverine environment. The mysid was found mainly in the bottom and mid-water layers, and observed density and mean length of drifting individuals increased with decreasing light levels, being highest at night. Similarly, density and mean length of drifting individuals increased with increasing turbidity. The effect of turbidity on density was particularly apparent during the night samples, suggesting further advantage to night-drifting individuals.

1. Introduction

The problem of invasions is widespread, covering species from insects to mammals in various ecosystems of all continents (WILLIAMSON, 1996). In the past 20 years, many aquatic species have extended their distributions (CARLTON and GELLER, 1993). In freshwaters the spread of crustaceans and fish are well documented (*e.g.* GARCÍA-BERTHOU, 2007; CHUCHOLL *et al.*, 2008). In recent years *Hemimysis anomala* G. O. SARS, 1907, a necto-benthic mysid shrimp native to the Black and Caspian Seas (PIENIMÄKI and LEPPÄKOSKI, 2004; HOLDICH *et al.*, 2006), has become one of the top invaders of the river systems of continental Europe (WITTMANN, 2007). The spread of the species was launched by the intentional introduction to sites in the former Soviet Union in order to enhance food supply for fish (ZHURAVEL, 1960). According to AUDZIJONYTE *et al.* (2008), the invasion of *H. anomala* has proceeded along two corridors: one via the Baltic Sea and further to the Rhine delta, probably from a population intentionally introduced to a Lithuanian water reservoir from the Dnieper River in 1960; the other from the Danube delta via the Danube and the Main-Danube Canal to the Rhine River delta. The first record of *H. anomala* in the Rhine occurred in 1997 (VAN DER VELDE *et al.*, 2000), five years after the opening of the Main-Danube Canal in 1992. Further spread of the species through the connections of the Rhine and other large rivers in Europe

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via canals and sea routes has been suggested (Leuven *et al.*, 2009). Accordingly, the species was recorded in the river Elbe, Czech Republic, in 2003, six years after its first occurrence in the Rhine (Horecký *et al.*, 2005).

H. anomala is essentially omnivorous with an increasing proportion of zooplankton in its diet in later life stages (Ketelaars *et al.*, 1999; Borcherding *et al.*, 2006). It is usually observed in swarms aggregating in rocky crevices, under stones and in the cavities of river banks, particularly where banks are reinforced (Salemaa and Hietalahti, 1993; Wittmann, 2007; Stubbington *et al.*, 2008). Previous reports of *H. anomala* populations have indicated a preference for lentic waters such as lakes, canals and river backwaters (Verslyke *et al.*, 2000; Dumont, 2006; Stubbington *et al.*, 2008). In such biotopes, the mysid rests on the bottom during light conditions at daytime and swims upwards during the night (Janas and Wysocki, 2005; Borcherding *et al.*, 2006). Nevertheless, precise information on the diurnal behaviour and vertical position of *H. anomala* in a riverine environment is lacking.

Non-native species may have adverse effects on structure and function of aquatic communities (Lasenby *et al.*, 1986; Dick and Platvoet, 2000). Thus, knowledge of their biology and ecology is of crucial importance in order to predict their impact and the likely success of any potential eradication programme (Simberloff, 2009). In the present study, the following assumptions of *H. anomala* in rivers were assessed: i) the species would spread upstream; ii) the species would exhibit the same diurnal behaviour as observed in lentic waters (Janas and Wysocki 2005; Borcherding *et al.*, 2006). To test the presence/absence and further spread of *H. anomala*, macroinvertebrate community structure was sampled four times per year from 2006 to 2008 at 896 sites scattered across the whole Czech Republic. Diurnal behaviour of *H. anomala* was assessed using drift net sampling performed weekly from July to September 2007 in the river Elbe, Czech Republic.

2. Methods

2.1. Study area and sampling

The distribution of *H. anomala* was assessed from samples of the macroinvertebrate community collected from 896 sites scattered across the Czech Republic. These sites were sampled at least twice a year from 2007 to 2008. Standard sampling methods for regular monitoring of running waters according to Water Framework Directive (WFD; 2000/60/ECC) requirements were used: these methods were the same as those used by Horecký *et al.* (2005) who recorded the first occurrence of the species in the Czech Republic.

Drift net sampling was carried out on the river Elbe, Czech Republic near Ústí nad Labem (distance from the source 322 km; 50°39′ N; 14°06′ E; Fig. 1). The river rises at 1,383 m above sea level. The Czech section of the river is 368 km long and has a catchment area of 51,394 km^2. At the study site the river is 100–150 m wide and canalized with its banks reinforced with rocks and concrete. Commercial shipping is frequent in this stretch of river. The long-term average during the study was 8 heavy-freight ships and 5 commercial passenger ships per day (The Elbe River Authority, unpubl. data).

Drift nets were deployed weekly from 12 July to 20 September 2007 (*i.e.* ten 24-h cycles). Three nets (opening 33 × 33 cm, 0.5 mm mesh size, 120 cm net length) were arranged on a 190 cm high frame in order to collect samples from the bottom (5–38 cm), mid-water (76–109 cm) and surface (147–180 cm) of the river. Frames and nets were deployed at the same position in the river on each occasion and held in position by weights. All the samples were collected 10 m from the river banks, where the average flow ranges from 0.6 to 0.8 $\mathrm{m \cdot s^{-1}}$ (data from the hydraulic model MIKE 21 C; see Horký *et al.*, 2008 for detail). Samples of drifting invertebrates were collected for 30 min once every four hours during the 24-h cycle (0000–0359, 0400–0759, 0800–1159, 1200–1559, 1600–1959, 2000–2359 hours). A flow meter (Global Flow Probe FP 201, Global Water Instrumentation, Inc., USA) was used to measure water velocity at the net openings at the beginning of each sampling period in order to quantify the volume of river water filtered. All material collected was preserved in 96% ethanol for laboratory processing. Organisms were identified, counted and total body length (TL) measured to the nearest 0.1 mm using

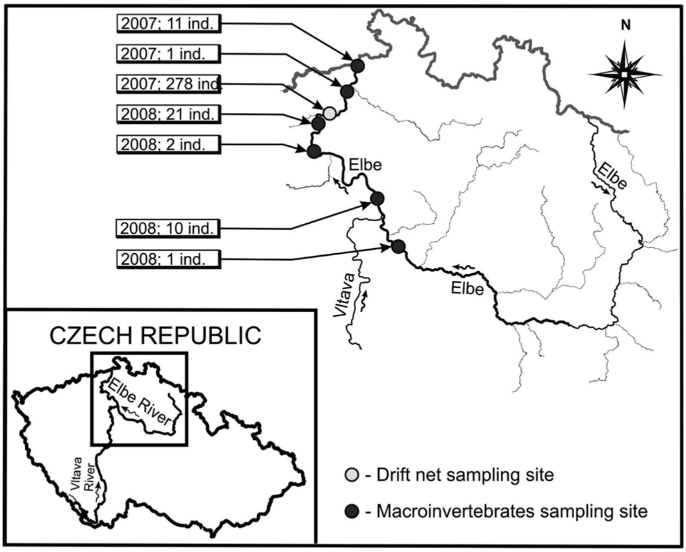

Figure 1. Map showing the location of the river stretch studied. Particular sampling sites with the year of the first occurrence and numbers of *Hemimysis anomala* caught are indicated.

a stereo microscope. Total body length of *H. anomala* was measured as the distance between the anterior tip of the carapace and the posterior margin of the telson (Pothoven *et al.*, 2007). Females were identified by the presence of a marsupium and reduced ventral appendages. Males were recognised by examining the length of the fourth pleopod, which is elongated.

2.2. Habitat measurement

Water temperature (°C), dissolved oxygen concentration (mg l^{-1}), conductivity (µS), pH, and turbidity (NTU) were measured using field meters (Oxi 196; pH/Cond 340i/SET; TURB 355 T; WTW, Germany). Light intensity at the surface (Ev) was measured using a SECONIC Super Zoom Master L-68 light meter (Seconic, Tokyo, Japan) during each drift sample collection. Measurements of the atmospheric pressure and the phase of the moon were made with a BAR 928 H remote weather station (Huger Electronics, Germany). The Elbe River Authority measured flow daily at a gauging station located within the study stretch.

2.3. Data analyses

The number of specimens collected per net per hour was estimated from direct counts, corrected for any deviation in sample duration or percent of net mouth submerged. Drift densities were calculated according to Allan and Russek (1985) as follows:

$$\mathrm{SDD} = \mathrm{N} \cdot \mathrm{V}$$

where SDD is the sample drift density expressed as the total number of individuals drifting past the sampling net per hour; N is the number of individuals per net per hour; V is the volume of water (m^3) filtered per net per hour, estimated from the area of the mouth of the net and water velocity at the net mouth. Sample drift density was estimated for every net and sampling interval separately.

2.4. *Statistical analyses*

Associations between the variables were tested using the Linear Mixed Model (LMM). Separate models were applied for the following dependent variables: sample drift density (LMM I) and total body length (LMM II). Where necessary, data were $\log_{10}$ transformed prior to analysis in order to meet requirements of normality and homoscedasticity. To account for repeated measurements across the period of observation, analyses were performed using a generalized mixed model with date (LMM I–II) as random factors, using PROC MIXED (SAS, version 9.1).

For the LMM I–II models, the fixed effects used were the class variables 'moon phase' (8 levels), 'sex' (juvenile, male, female), 'water layer' (bottom, mid-water, surface) and 'light interval' (day, night, twilight), and the continuous variables 'turbidity' (range 3.89–38.34 NTU), water temperature' (15.5–25.2 °C), 'water flow' (84–151 $m^3 s^{-1}$), 'atmospheric pressure' (992–1033 hPa), 'conductivity' (332–425 µS), 'light intensity' (0–15.1 Ev), and 'dissolved oxygen' (5.5–12.9 mg l^{-1}). The significance of each fixed effect (including interactions) in the analyses was assessed using an F-test, upon sequential dropping of the least significant effect, starting with a full model (backward elimination). Fixed effects and interactions that were not statistically significant are not discussed further. In these analyses with unbalanced designs and more than one effect, the arithmetic mean for a group may not accurately reflect the response for that group, since it does not take other effects into account. Therefore, the least-squares-means (LSMEAN) are reported rather than arithmetic means. LSMEAN (further referred to as 'adjusted means') are, in effect, within-group means appropriately adjusted for the other effects in the model. Adjusted probabilities (Adj P) were computed for each class; differences between classes were tested using a *t*-test. For multiple comparisons, the Tukey-Kramer adjustment was used. Associations between the dependent variable and other continuous variables were estimated by fitting a random coefficient model using PROC MIXED as described by Tao *et al.* (2002). Using this random coefficient model, we calculated predicted values for the dependent variable and plotted them against the continuous variable with predicted regression lines. The degrees of freedom were calculated using the Kenward-Roger method (Kenward and Roger, 1997).

3. Results

By 2008 *H. anomala* was recorded throughout a 129 km long stretch of the Elbe from the border with Germany to just north of the confluence with the Vltava River. However, *H. anomala* appears to be spreading progressively through the river Elbe. During the study *H. anomala* was documented at six monitoring sites (distance from the source 229, 255, 297, 317, 337 and 358 km respectively; Fig. 1). *H. anomala* was not found on the other sites which were surveyed and was only found in the Elbe.

The final LMM model for drift density (LMM I) contained the fixed factors light interval, net position and turbidity nested within light interval; for the length of individuals drifting (LMM II) the model contained turbidity and light interval (for details of the models see Table 1). A total of 180 drift net samples were collected during ten 24-h cycles in 2007. Den-

Table 1. Type 3 tests of fixed effects for final significant Linear Mixed Models (LMM; significant effects as well as their interactions are provided).

Effect	Num DF	Den DF	F	P <
For LMM I – sample drift density				
Light interval	2	178	15.58	0.0001
Water layer	2	169	5.08	0.0072
Turbidity (light interval)	3	172	15.39	0.0001
For LMM II – total body length				
Turbidity	1	278	11.18	0.0009
Light interval	2	278	7.8	0.0010

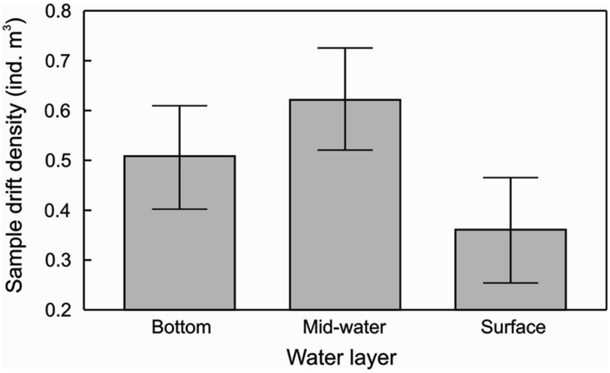

Figure 2. Drift density of *Hemimysis anomala* at different depths. Values are adjusted means ± S.E. of $\log_{10}$ transformed data.

sities of drifting *H. anomala* varied from 0 to 162.8 ind m^{-3} (mean 27.2 ind m^{-3}) comprising an average of 36.3% males, 13% females and 50.7% juveniles, indicating that the species is reproducing in the river Elbe. Juveniles varied in length from 1.5 to 7.0 mm (mean 4.1 mm; n = 141). Adults were 4.7–10.5 mm long, with females (mean 7.3 mm; range 4.7–10.5 mm; n = 36) slightly exceeding the size of males (mean 7.0 mm; range 4.8–10.2 mm; n = 101).

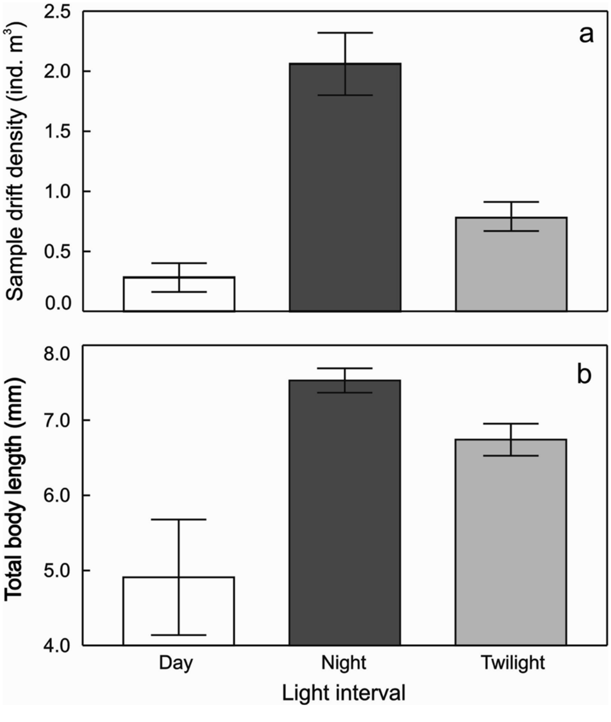

Figure 3. Variation in (a) drift density and (b) mean total body length of *Hemimysis anomala* during different light intervals. Values are adjusted means ± S.E. of $\log_{10}$ transformed data.

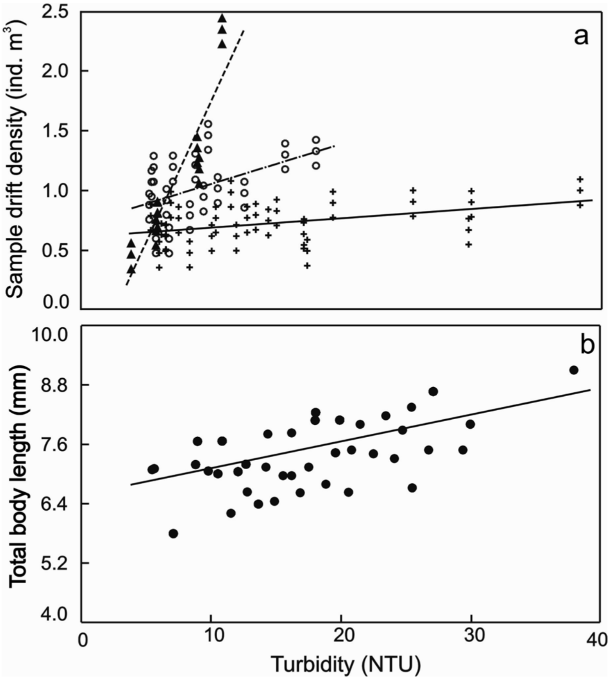

Figure 4. The relationship between turbidity and (a) drift density across light intervals: day (+, ——), night (▲, ----), twilight (○, - - -); (b) total body length of *Hemimysis anomala*. Predicted values are from $\log_{10}$ transformed data. The fitted curves were: (a) day $y = 0.0094x + 0.6271$ ($r^2 = 0.15$), night $y = 0.2853x - 0.6734$ ($r^2 = 0.84$), twilight $y = 0.0408x - 0.7143$ ($r^2 = 0.23$) and (b) $y = 0.0054x + 0.6574$ ($r^2 = 0.31$).

Drift samples collected from different depths revealed that *H. anomala* was found mainly in the mid-water layer of the water column, with significantly more individuals in the bottom and mid-water than the surface (Fig. 2; bottom vs. mid-water Adj. $P > 0.05$; bottom vs. surface Adj. $P < 0.05$; mid-water vs. surface Adj. $P < 0.05$). The density and length of drifting individuals increased with decreasing light levels, being highest at night (Fig. 3a, b; all Adj. $P < 0.05$). Furthermore, the density of *H. anomala* (Fig. 4a) and the length of drifting individuals (Fig. 4b) increased with increasing turbidity. Detailed analyses revealed that only the slope of night density significantly differed from zero ($P < 0.0001$) and from the other light intervals (day and twilight; both $P < 0.0001$). The other environmental variables tested were not found to have a significant effect on *H. anomala* behaviour.

4. Discussion

It has been suggested that *H. anomala* dispersal is largely via shipping (WITTMANN, 2007), as it has been documented from bays, estuaries and inland waters around the world (CARLTON and GELLER, 1993). Further dispersal of invasive amphipods is facilitated by drifting (VAN RIEL *et al.*, 2011). In the Czech Republic, the species was found only within the lower section of the Elbe, which was characterized by the presence of harbours and frequent shipping. The occurrence of juveniles and gravid females suggests a reproducing population in the river itself; however, the occurrence of these individuals may be the result of reproducing populations upstream of the sampling sites as well. The riverbed of the Elbe has been narrowed and deepened, confirming the suggestion of STUBBINGTON *et al.* (2008) that the species is largely confined to anthropogenic habitats. By 2008 the mysid had progressively spread through the lower 129 km of the Czech section of the Elbe from the border with Germany. Further spread of the species via ship transport may be possible; a further 80 km long stretch of the Elbe upstream of the last point of mysid capture is regularly used by large ships. Although not yet recorded, it is possible that *H. anomala* will spread to the river Vltava, since shipping frequently travels between the Vltava and the Elbe.

The present study shows that in a riverine environment with relatively high current speed (around 0.6–0.8 $m \cdot s^{-1}$) *H. anomala* displays the same diel activity pattern observed in lentic waters (JANAS and WYSOCKI, 2005; BORCHERDING *et al.*, 2006). *H. anomala*, which has been described as a weak swimmer (WITTMAN, 2007), may persist in such a fluvial environment by utilizing crevices in rocky substrates, particularly in areas where anthropogenic structures provide refugia (STUBBINGTON *et al.*, 2008). The number of drifting individuals peaked during the night. Several authors have described sheltering behaviour of the species during the day (KETELAARS *et al.*, 1999; BORCHERDING *et al.*, 2006; DUMONT, 2006), when it remains hidden in crevices between boulders or under hard surfaces (SCHLEUTER *et al.*, 1998; HOLDICH *et al.*, 2006). Access to refugia could also be the reason why *H. anomala* was found predominantly in the bottom and mid-water layers in the river Elbe.

Vertical migration patterns of crustaceans have been shown to be an adaptive behaviour to avoid predatory fish (GHAN *et al.*, 1998; THYS and HOFFMAN, 2005). Behavioural patterns of *H. anomala* also appear to be adapted to minimize predator encounter rates. Decreased light levels and/or increased turbidity may offer shelter for mysids, by reducing the feeding success of fish (KIRK, 1981). Migration of nekton in turbid waters has rarely been studied: our findings suggest that turbidity is an important factor regulating the behaviour of mysids in rivers. In the present study, the number of drifting *H. anomala* increased with turbidity and this trend was particularly apparent during night. This aspect is probably an outcome of a two major factors: predator avoidance and increased resource availability. Visually oriented fish are able to utilize even very low light intensities, to increase their predation success (*e.g.* HOBSON, 1965; FRASER and METCALFE, 1997). Thus, turbidity can be important even during the night, as it further decreases the probability of being detected by predators. Correspondingly, diel movements and home range size of ide, *Leuciscus idus* L., a potential mysid predator tracked within the same stretch of the river Elbe, increased with increased turbidity presumably in response to reduced foraging success (KULÍŠKOVÁ *et al.*, 2009). The observed *H. anomala* behaviour might also be a consequence of food availability (HEAD *et al.*, 1985; BORCHERDING *et al.*, 2006). During night we may suggest higher abundance of preferred prey (zooplankton) which may also reflect higher turbidity levels.

KETELAARS *et al.* (1999) suggested that *H. anomala* adults and juveniles display spatial segregation: such behaviour was recorded in the present study. Smaller individuals (*i.e.* juveniles) were found in drift samples during the day and at lower turbidity, whereas larger individuals (*i.e.* adults) were found in drift samples during the night and under conditions of increased turbidity. KETELAARS *et al.* (1999) suggested that the activity of juveniles during less favourable conditions was the result of a combination of avoiding cannibalism by larger conspecifics and being less vulnerable to fish predation.

In summary, *H. anomala* have the potential to become wide spread where introduced to riverine environments, although its occurrence may be underestimated by common monitoring practices. The results of this study indicate that more precise evaluation of dispersal of the species and its impact in riverine environments could be achieved by night sampling with drift nets.

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6. References

Allan, J. D. and E. Russek, 1985: The quantification of stream drift. – Can. J. Fish. Aquat. Sci. **42**: 210–215.

Audzijonyte, A., K. J. Wittmann and R. Väinölä, 2008: Tracing recent invasions of the Ponto-Caspian mysid shrimp *Hemimysis anomala* across Europe and to North America with mitochondrial DNA. – Divers. Distrib. **14**: 179–186.

Borcherding, J., S. Murawski and H. Arndt, 2006: Population ecology, vertical migration and feeding of the Ponto-Caspian invader *Hemimysis anomala* in a gravel-pit lake connected to the River Rhine. – Freshwat. Biol. **51**: 2376–2387.

Carlton, J. T. and J. B. Geller, 1993: Ecological roulette: the global transport of nonindigenous marine organisms. – Science **261**: 78–82.

Chucholl, C., H. B. Stich and G. Maier, 2008: Aggressive interactions and competition for shelter between a recently introduced and an established invasive crayfish: *Orconectes immunis* vs. *O. limosus*. – Fundam. Appl. Limnol. **172**: 27–36.

Dick, J. T. A. and D. Platvoet, 2000: Invading predatory crustacean *Dikerogammarus villosus* eliminates both native and exotic species. – Proc. R. Soc. Lond., Ser. B: Biol. Sci. **267**: 977–983.

Dumont, S., 2006: A new invasive species in the north-east of France, *Hemimysis anomala* G. O. Sars, 1907 (Mysidacea). – Crustaceana **79**: 1269–1247.

Fraser, N. H. C. and N. B. Metcalfe, 1997: The costs of becoming nocturnal: feeding efficiency in relation to light intensity in juvenile Atlantic salmon. – Functional Ecology **11**: 385–391.

García-Berthou, E., 2007: The characteristics of invasive fishes: what has been learned so far? – J. Fish Biol. **71**: 33–55.

Ghan, D., K. D. Hyatt and J. D. McPhail, 1998: Benefits and costs of vertical migration by the freshwater copepod *Skistodiaptomus oregonensis*: testing hypotheses through population comparison. – Can. J. Fish. Aquat. Sci. **55**: 1338–1349.

Head, E. J. H., L. Harris and C. Abou Debs, 1985: Effect of daylength and food concentration on in situ diurnal feeding rhythms in Arctic copepods. – Mar. Ecol. Prog. Ser. **24**: 281–288.

Hobson, E. S., 1965: Diurnal-nocturnal activity of some inshore fishes in the Gulf of California. – Copeia **1965**: 291–302.

Holdich, D., S. Gallagher, L. Rippon and P. Harding, 2006: The invasive Ponto-Caspian mysid, *Hemimysis anomala*, reaches the UK. – Aquat. Inv. **1**: 4–6.

Horecký, J., F. Šporka and E. Stuchlík, 2005: First record of *Hemimysis anomala* SARS (Crustacea: Mysidacea) from Czech stretch of Elbe river (Czech Republic). – Lauterbornia **55**: 89–91.

Horký, P., O. Slavík and L. Bartoš, 2008: A telemetry study on the diurnal distribution and activity of adult pikeperch *Sander lucioperca* (L.) in a riverine environment. – Hydrobiologia **614**: 151–157.

Janas, U. and P. Wysocki, 2005: *Hemimysis anomala* G. O. Sars, 1907 (Crustacea, Mysidacea) – first record in the Gulf of Gdańsk. – Oceanologia **47**: 405–408.

Kenward, M. G. and J. H. Roger, 1997: Small sample inference for fixed effects from restricted maximum likelihood. – Biometrics **53**: 983–997.

Ketelaars, H. A. M., F. E. Lambregts-van de Clundert, C. J. Carpentier, A. J. Wagenvoort and W. Hoogenboezem, 1999: Ecological effects of the mass occurrence of the Ponto-Caspian invader, *Hemimysis anomala* G. O. Sars, 1907 (Crustacea: Mysidacea), in a freshwater storage reservoir in the Netherlands, with notes on its autecology and new records. – Hydrobiologia **394**: 233–248.

Kirk, J. T. O., 1981: Estimation of the scattering coefficient of natural waters using underwater irradiance measurements. – Aust. J. Mar. Freshw. Res. **32**: 533–539.

Kulíšková, P., P. Horký, O. Slavík and J. I. Jones, 2009: Factors influencing movement behaviour and home range size in ide *Leuciscus idus*. – J. Fish Biol. **74**: 1269–1279.

Lasenby, D. C., T. G. Northcote and M. Fürst, 1986: Theory, practice, and effects of *Mysis* introductions to North American and Scandinavian lakes. – Can. J. Fish. Aquat. Sci. **43**: 1277–1284.

Leuven, R. S. E. W., G. Van der Velde, I. Baijens, J. Snijders, Ch. Van der Zwart, H. J. R. Lenders and A. Bij de Vaate, 2009: The river Rhine: a global highway for dispersal of aquatic invasive species. – Biol. Invasions **11**: 1989–2008.

Pienimäki, M. and E. Leppäkoski, 2004: Invasion pressure on the Finnish Lake District: invasion corridors and barriers. – Biol. Invasions **6**: 331–346.

Pothoven, S. A., I. A. Grigorovich, G. L. Fahnenstiel and M. D. Balcer, 2007: Introduction of the Ponto-Caspian bloody-red mysid *Hemimysis anomala* into the Lake Michigan Basin. – J. Great Lakes Res. **33**: 285–292.

Salemaa, H. and V. Hietalahti, 1993: *Hemimysis anomala* G. O. Sars (Crustacea: Mysidacea) – Immigration of a Pontocaspian mysid into the Baltic Sea. – Ann. Zool. Fenn. **30**: 271–276.

Schleuter, A., H. P. Geissen and K. J. Wittmann, 1998: *Hemimysis anomala* G. O. Sars, 1907 (Crustacea, Mysidacea), eine euryhaliene pontokaspische Schwebgarnale in Rhein und Neckar. Erstnachweis für Deutschland. – Lauterbornia **32**: 67–71.

Simberloff, D., 2009: We can eliminate invasions or live with them. Successful management projects. – Biol. Invasions **11**: 149–157.

Stubbington, R., Ch. Terrell-Nield and P. Harding, 2008: The first occurrence of the ponto-caspian invader, *Hemimysis anomala* G. O. Sars, 1907 (Mysidacea) in the U.K. – Crustaceana **81**: 43–55.

Tao, J., R. Little, M. Patetta, C. Truxillo and R. Wolfinger, 2002: Mixed the SAS System Course Notes. – SAS Institute Inc., Carry, NC, USA.

Thys, I. and L. Hoffman, 2005: Diverse responses of planktonic crustaceans to fish predation by shifts in depth selection and size at maturity. – Hydrobiologia **551**: 87–98.

Van der Velde, G., S. Rajagopal, B. Kelleher, I. B. Muskó and A. Bij de Vaate, 2000: Ecological impact of crustacean invaders: general considerations and examples from the Rhine River. – Crustac. Issues **12**: 3–33.

Van Riel, M. C., G. Van Der Velde and A. Bij De Vaate, 2011: Dispersal of invasive species by drifting. – Current Zoology **57**: 818–827.

Verslycke, T., C. Janssen, K. Lock and J. Mees, 2000: First occurrence of the Ponto-caspian invader *Hemimysis anomala* (Sars, 1970) in Belgium (Crustacea: Mysidacea). – Belg. J. Zool. **130**: 157–158.

Williamson, M., 1996: Biological Invasions. – Chapman and Hall, London.

Wittmann, K. J., 2007: Continued massive invasion of Mysidae in the Rhine and Danube river systems, with first records of the order Mysidacea (Crustacea: Malacostraca: Peracarida) for Switzerland. – Rev. Suisse Zool. **114**: 65–86.

Zhuravel, P. A., 1960: The mysid *Hemimysis anomala* Sars (Crustacea Malacostraca) in the Dnieper water reservoir and its feeding value for fishes. – Zool. Zh. **39**: 1571–1573.

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CHAPTER IV

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Young-of-the-year (YOY) assemblage sampling as a tool for assessing the ecological quality of running waters

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Summary

The aim of this study was to test young-of-the-year (YOY) assemblage applicability for the ecological quality assessment on the basis of samples from 55 sites scattered across the entire Czech Republic. European Fish Index (EFI) values estimated with YOY and adults were positively correlated, indicating that YOY and adult sampling is comparable. YOY bias the EFI toward significantly lower values, however, adults also lead to underestimated EFI values on some occasions. Relative differences between EFI YOY and EFI adults show almost normal distributions and indicate that there is no detectable systematic bias caused by environmental conditions or intensity of sampling. As shown in the Elbe River example, the reason for lower EFI YOY values could be found in a longer period of detection of evident changes in adult fish assemblages, reflecting longevity of adult fishes or regular stocking. YOY sampling was suggested as a useful method for assessing river ecological quality, with the ability to provide a sensitive response to the habitat structure regardless of the effect of stocking or river size.

Introduction

The Water Framework Directive (WFD; 2000/60/ECC) requirements are to classify ecological quality of all waters by using different biological quality elements. Fish represent one of these elements, being usually assessed by the unifying European Fish Index (EFI) in running waters (Schmutz et al., 2007). The EFI methodology is based on the sampling of the entire fish assemblage, with emphasis on the adults. This paper describes a different approach based on the young-of-the-year (YOY) sampling that was established in the Czech Republic.

Young-of-the-year of European freshwater fishes occupy species-specific habitats reflecting their life-history requirements (Kryzhanovsky, 1949). Accordingly, YOY sampling was proved as a tool defining the availability of spawning/nursery habitats at a site (Oberdorff and Hughes, 1991; Copp, 1992) as well as the ecological function and integrity of riverine systems (e.g. Copp, 1989b, 1992; Oberdorff et al., 1993; Garner, 1995; Schiemer et al., 2003). Young-of-the-year sampling provides a proper response to population dynamics, since year-class strength is mainly affected by events that occur in the fishes' early life (Balon, 1984). Thus, existence of a suitable spawning/nursery habitat and food availability could be of the utmost importance (Garner, 1996; Lightfoot and Jones, 1996). YOY are also easier to collect in large rivers than adult fish, and therefore are thought to better estimate the actual fish assemblage structure at specific sites (Cattanéo, 2005).

The aim of the present study was to test whether sampling of YOY is applicable for assessing the ecological quality in running waters. To address this issue, the relationship between EFI values estimated with YOY and EFI values estimated with adults was described on the basis of samples from 55 sites scattered across the entire Czech Republic.

Materials and methods

Study sites

All sampling sites were selected as representative of specific river stretches (Fig. 1); their basic characteristics are shown in Table 1. The 55 sites used for comparison of EFI values estimated with YOY and adults included medium to large rivers (distance from the source range 30–407 km) and pristine to human-influenced rivers. These sites were sampled once during the 2003–2007 period.

The seven sites used for long-term analyses were distributed along the longitudinal profile of the Elbe River (distance from the source range 61–366 km). These sites were sampled annually (one sample per year) from 1995 to 2006, according to the requirements of the International Commission for the Protection of the Elbe River.

Sampling procedures

All sampling procedures were conducted with the help of electrofishing. Adult as well as YOY assemblages were sampled separately on each site during the same day. A partial sampling procedure was applied, covering all types of habitats to obtain a representative sample of the site. Sampling area borders were determined with help of the portable GPS receiver (GPS map 76S, Garmin Ltd.). The sampling areas considered ranged from 100 m^2 to 10 000 m^2, depending on the river size and habitat heterogeneity. All samplings were undertaken during late summer, to assure efficiency of the YOY sampling (Copp, 1989a).

Electrofishing of adults was conducted by wading or by boat, depending on the river size (backpack FEG 1500 or stationary FEG 8000 pulsed-DC electrofishing units, EFKO-Germany; maximum output 650 V, 10 A; 50 cm ø circle anode, 6 mm mesh size). Fish were identified to species and immediately released at the site of capture.

Electrofishing of YOY was conducted by wading near the bank in an upstream direction, regardless of the river size (back-pack pulsed-DC electrofishing unit LENA, Bednář,

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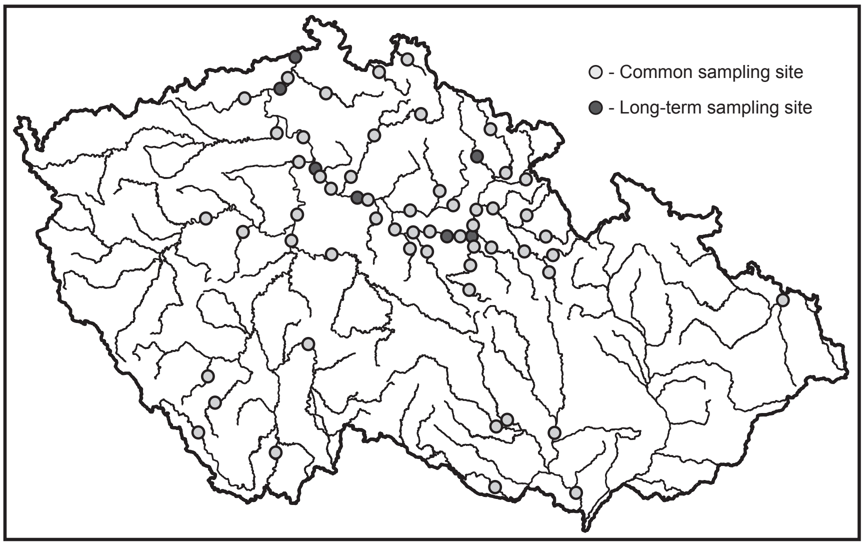

Fig. 1. Map of sampling sites in the Czech Republic river network (light dots = common sampling sites; dark dots = long-term sampling sites)

Czech Republic; max. output 225–300 V, 6 A; oval anode 42 × 20 cm, 2 mm mesh size). Although point abundance and continuous sampling of YOY are comparable in terms of qualitative analyses, continuous sampling was preferred in order to allow quantitative interpretation of results (Janáč and Jurajda, 2007). Most fish were identified to species and immediately released at the site of capture. Specimens that could not be reliably identified were fixed in 4% formaldehyde solution for laboratory identification.

Data analyses

Data on the extent between initial and terminal borders of sampling area were transferred from GPS to PC, and analyzed with the help of the Map Source Ver. 5.3 (Garmin Ltd.) in order to estimate the size of the sampling area. European fish index values for each site and each sampling method (adult and YOY) were computed using the EFI software (FAME Consortium, 2004). European fish index values based on sampling of adults were labelled 'EFI adult' and EFI values based on sampling of YOY were labelled 'EFI YOY'. Relative difference between 'EFI adult' and 'EFI YOY' at particular sites was labelled 'adult YOY difference' (adult YOY difference=EFI adult−EFI YOY). Data for the long-term analyses were grouped in subsequent three-years intervals (1995–1997, 1998–2000, 2001–2003, 2004–2006) and this factor was labelled 'time interval'.

Statistical analyses

Associations between the variables were tested using the General Linear Mixed Model (GLMM). Separate models were applied for the dependent variables: EFI adult (GLMM I; comparison of EFI YOY and EFI adult), all EFI values (GLMM II; comparison of EFI YOY and EFI adult), adult YOY difference (GLMM III; comparison of EFI YOY and EFI adult), number of species in YOY assemblage (GLMM IV; long-term analyses) and number of species in adult fish assemblage (GLMM V; long-term analyses). The data were tested for normality (Kolmogorov–Smirnov tests for normal distribution) and square root transformed before GLMM analyses when needed. To account for the bias caused by different characteristics of the sampling sites, all analyses were performed using mixed model analysis, with the sampling site as a random factor using PROC MIXED (SAS, version 9.1). Interaction between sampling site and year was used as an additional random factor in the long-term analyses (GLMM IV–V). The fixed effects were the continuous variable 'EFI YOY' for GLMM I, class 'sampling method' for GLMM II, continuous variables altitude, slope, distance from the source, overall fished area or relative difference in fished area between YOY and adults for GLMM III, and class 'time interval' for GLMM IV and V. The significance of each fixed effect (including interactions) in the mixed GLMM models was assessed by the F-test with sequential discarding of the least significant effect, starting with a full model. Least-squares-means (further 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. Associations between the dependent variable and other continuous variables were estimated by fitting a random coefficient model using PROC MIXED as described by Tao et al. (2002). The degrees of freedom were calculated using the Kenward–Roger method (Kenward and Roger, 1997).

Results

A total of 14 633 fishes (7 178 adults; 7 455 YOY) representing 35 species (35 species of adults; 31 species of YOY respectively) from 55 sites (Table 2) were used to compare the EFI values estimated with YOY and adults.

A total of 17 065 fishes (10 828 adults, 6237 YOY, respectively) representing 31 species (31 adult species, 26 YOY species, respectively) were used for long-term analyses of the seven sites on the Elbe River.

The four most numerous species were bleak *Alburnus alburnus* (L.), chub *Leuciscus cephalus* (L.), roach *Rutilus rutilus* (L.), and gudgeon *Gobio gobio* (L.). The overall species-specific densities and total number of specimens by species are shown in Table 3 (adults) and Table 4 (YOY). Details of statistical models are presented in Table 5.

Table 1
Characteristics of particular sampling sites: 'Distance' = distance from source; 'Channelization', 'Vegetation' and 'Habitat' = degree of human-induced alterations of these characteristics; 1 – no, 2 – low, 3 – medium, 4 – heavy. Long-term sampling sites indicated with asterisks

Site	Catchment	Geology	Altitude (m)	Distance (km)	Channelization	Vegetation	Habitat
1	Danube	Calcareous	168	129	2	3	3
2	Danube	Siliceous	392	39	2	2	2
3	Danube	Siliceous	191	159	2	1	1
4	Danube	Siliceous	150	248	3	2	3
5	Danube	Siliceous	167	146	2	2	1
6	Elbe	Siliceous	156	238	4	3	3
7	Elbe	Siliceous	181	209	3	3	3
8	Elbe	Siliceous	729	45	2	2	2
9	Elbe	Siliceous	185	272	3	2	3
10	Elbe	Siliceous	130	72	4	4	4
11	Oder	Siliceous	197	100	2	3	3
12	Elbe	Siliceous	219	110	2	2	3
13	Elbe	Siliceous	235	89	3	2	2
14	Elbe	Siliceous	186	137	4	3	4
15	Elbe	Siliceous	281	92	3	3	4
16	Elbe	Siliceous	349	56	3	2	2
17	Elbe	Siliceous	228	74	3	2	3
18	Elbe	Siliceous	204	133	4	4	4
19	Elbe	Siliceous	267	102	3	4	3
20	Elbe	Siliceous	413	59	3	2	3
21	Elbe	Siliceous	249	82	4	3	4
22	Elbe	Siliceous	324	31	2	3	3
23	Elbe	Siliceous	253	75	1	2	1
24	Elbe	Siliceous	297	52	2	2	2
25	Elbe	Siliceous	262	73	2	2	1
26	Elbe	Siliceous	411	51	2	2	3
27	Elbe	Siliceous	365	35	2	1	2
28	Elbe	Siliceous	275	39	2	1	2
29	Elbe	Siliceous	252	88	3	2	3
30	Elbe	Siliceous	237	93	4	3	4
31	Elbe	Siliceous	490	47	2	2	3
32	Elbe	Siliceous	218	95	3	3	3
33	Elbe	Siliceous	198	63	4	3	4
34	Elbe	Siliceous	269	30	2	2	1
35	Elbe	Calcareous	192	70	3	3	3
36	Elbe	Siliceous	297	34	3	2	2
37	Elbe	Calcareous	192	70	2	2	3
38	Elbe	Siliceous	434	31	2	1	3
39	Elbe	Siliceous	233	76	2	3	3
40	Elbe	Siliceous	268	39	2	2	2
41	Elbe	Siliceous	325	47	1	2	2
42	Elbe	Siliceous	254	80	1	2	1
43	Elbe	Siliceous	169	140	2	3	3
44	Elbe	Siliceous	246	38	4	3	4
45	Elbe	Siliceous	221	33	2	3	3
46	Elbe	Siliceous	225	60	2	2	3
47	Elbe	Siliceous	426	46	2	2	2
48	Elbe	Siliceous	355	51	1	2	2
49	Elbe	Siliceous	253	75	3	2	3
50	Elbe	Siliceous	392	48	1	2	1
51	Elbe	Siliceous	224	110	2	3	2
52	Elbe	Siliceous	136	307	2	3	2
53	Elbe	Siliceous	171	372	3	3	3
54	Elbe	Siliceous	188	283	3	3	4
55	Elbe	Siliceous	162	407	2	2	2
56*	Elbe	Siliceous	341	61	1	2	1
57*	Elbe	Siliceous	220	117	2	2	2
58*	Elbe	Siliceous	211	140	4	4	4
59*	Elbe	Siliceous	158	253	4	3	4
60*	Elbe	Siliceous	174	216	3	3	3
61*	Elbe	Siliceous	125	345	2	3	2
62*	Elbe	Siliceous	121	366	3	3	3

Comparison of EFI YOY and EFI adults

Although European fish index values estimated with YOY and adults are positively correlated, indicating that YOY and adult sampling are comparable (Fig. 2); YOY bias EFI toward significantly lower values (Fig. 3; Tukey: $P < 0.02$). Accordingly, despite the similar trend of a number of sites in particular quality classes (Fig. 4), YOY classify a higher numbers of sites into the poor and bad ecological status. Nevertheless, this trend is not a general rule valid at all sites. Histogram of relative differences between EFI YOY and EFI adults show almost normal distributions, indicating that adults underestimate EFI values on some occasions as well

Table 2
Fish species identified during surveys, their Latin and common names as well as abbreviations used in the study

Code	Latin name	Common name
Ab	*Abramis brama* (L. 1758)	Common bream
Ap	*Alburnoides bipunctatus* (Bloch, 1782)	Schneider
Aa	*Alburnus alburnus* (L. 1758)	Bleak
An	*Ameirus nebulosus* (Lesueur, 1819)	Brown bullhead
Ag	*Anguilla anguilla* (Linnaeus, 1758)	European eel
Au	*Aspius aspius* (Linnaeus, 1758)	Asp
Br	*Barbatula barbatula* (Linnaeus, 1758)	Stone loach
Bb	*Barbus barbus* (Linnaeus, 1758)	Barbel
Bj	*Blicca bjoerkna* (L. 1758)	Silver bream
Ca	*Carassius carassius* (Linnaeus, 1758)	Crucian carp
Cp	*Carassius gibelio* (Bloch, 1782)	Prussian carp
Cn	*Chondrostoma nasus* (Linnaeus, 1758)	Common nase
Cg	*Cottus gobio* (Linnaeus, 1758)	Bullhead
Ci	*Ctenopharyngodon* idella (Valenciennes, 1844)	Grass carp
Cc	*Cyprinus carpio* (Linnaeus, 1758)	Common carp
El	*Esox lucius* (Linnaeus, 1758)	Northern pike
Gg	*Gobio gobio* (Linnaeus, 1758)	Gudgeon
Gc	*Gymnocephalus cernus* (Linnaeus, 1758)	Ruffe
Lc	*Leuciscus cephalus* (Linnaeus, 1758)	Chub
Li	*Leuciscus idus* (Linnaeus, 1758)	Ide
Ll	*Leuciscus leuciscus* (Linnaeus, 1758)	Dace
Lt	*Lota lota* (Linnaeus, 1758)	Burbot
Pf	*Perca fluviatilis* (Linnaeus, 1758)	Perch
Pp	*Phoxinus phoxinus* (Linnaeus, 1758)	Minnow
Pm	*Proterorhinus marmoratus* (Pallas, 1814)	Tubenose goby
Pr	*Pseudorasbora parva* (Temminck & Schlegel, 1846)	Topmouth gudgeon
Rs	*Rhodeus sericeus* (Pallas, 1776)	Bitterling
Rr	*Rutilus rutilus* (Linnaeus, 1758)	Roach
St	*Salmo trutta fario* (Linnaeus, 1758)	Brown trout
Se	*Scardinius erythrophthalmus* (Linnaeus, 1758)	Rudd
Sg	*Silurus glanis* (Linnaeus, 1758)	Wels catfish
Sl	*Sander lucioperca* (Linnaeus, 1758)	Pikeperch
Th	*Thymallus thymallus* (Linnaeus, 1758)	Grayling
Tt	*Tinca tinca* (Linnaeus, 1758)	Tench
Vv	*Vimba vimba* (Linnaeus, 1758)	Vimba bream

(Fig. 5). Furthermore, relative differences between EFI YOY and EFI adults do not vary with altitude, slope, distance from the source, overall fished area or relative difference in fished area between YOY and adults samplings, indicating that there is no detectable systematic bias caused by environmental conditions or intensity of sampling.

Long-term analyses

In the Elbe River, the number of successfully reproducing species decreased significantly from 1995 to 2006 (Fig. 6; Tukey: $P < 0.01$). In contrast, the number of species in the adult fish assemblage did not show any significant trends across this time span (GLMM V; $P > 0.05$).

Discussion

We found that sampling YOY and adults is comparable in terms of assessing the ecological quality of running waters using the EFI method. Providing that the YOY assemblage mirrors the reproductive success of local adult fish species with a sensitive response to the habitat quality (e.g. Copp, 1992) and ability to identify degraded riverine environments (Oberdorff and Hughes, 1991), sampling YOY could be suggested as a valuable tool for WFD purposes. However, despite the positive correlation of EFI values estimated with YOY and adults, YOY bias the EFI toward lower values. As shown in the Elbe River example in the present study, the reason could be in the longer period of detection of evident changes in the adult fish assemblage, reflecting the longevity of adult fishes. Even a decrease in the reproductive success does not have to be followed by the same trend in the adult fish assemblage, suggesting that stocking is of importance. In the Czech Republic, stocking is widespread and regular, with the ability to maintain adult fish assemblages at a certain level. The Czech Anglers Union stock over 1400 tonnes of 30 species per year in almost all streams and rivers; stocking of 22 species, including coarse fishes like asp *Aspius aspius* L. or barbel *Barbus barbus* L. is frequent (Czech Anglers Union, unpubl. data). As was also shown on the Great Ouse River where regulation caused local extinction (Maitland and Lyle, 1991) as well as low recruitment of some fish species (Copp, 1990, 1992; Copp and Mann, 1993), restocking programs are not the appropriate solution. The reason is that stocking responds only to the symptoms and does not address the root of the problem, which lies in the lack of suitable spawning, nursery and refuge habitats (Copp, 1992). Young-of-the-year sampling is able to detect this problem as well as minimize the effects of stocking on the ecological quality assessment.

However, the narrow ecological requirements of YOY that provide sensitive responses to the habitat quality (Copp, 1992) also represent the main disadvantage of their sampling. Young-of-the-year assemblages are more vulnerable to hydrological events such as floods or droughts than adults (Schlosser, 1985; Cattanéo et al., 2001; Freeman et al., 2001). These fish-hydrology relationships are species-specific, as responses to flow variability depend on their reproductive strategies (Cattanéo, 2005). The first strategy that is close to the periodic life history (Winemiller and Rose, 1992) or r strategy (Pianka, 1970) is represented by predominantly lithophilic, highly fecund, late and fractional spawners with small eggs. These fish species are favoured by high water flows and duration of floods during their growth period (Cattanéo, 2005). In addition to the general adaptation to the water flow is that fractional spawners are thought to be better adapted to a single disturbance event (Pont et al., 1995). The second strategy, one that is close to equilibrium (Winemiller and Rose, 1992) or K strategy (Pianka, 1970), is represented by medium fecund, early and one-batch spawners having medium or large eggs. These fish species are adversely affected by high spawning flows (Cattanéo, 2005) and are assumed to be found predominantly in stable habitats. The above mentioned fish-hydrology relationships should be considered in assessing the ecological quality of running waters, although they are generally weak (i.e. <10% of explained variability). To avoid hydrological bias, relative abundance data less sensitive to flow variability should be considered preferable as a metric to describe assemblage structure (Cattanéo et al., 2002). Also, repeated sampling at one site in consecutive years could be recommended. Another drawback of YOY sampling is that it does not estimate the age structure of fish populations. The WFD requires age structure determination as an indication of a reproduction failure or the development of any particular species. However, YOY sampling provides information on reproduction success itself. Furthermore, the numbers of YOY determine the strength of subsequent year-classes, being fundamentally important to population dynamics (Houde, 1994). Thus, repeated and sufficient natural reproduction could be suggested as a good

Table 3
Species-specific density (ind m^{-2}) of adult fishes at sampling sites. Long-term sampling sites indicated with asterisks (abundances at these sites are considered for the whole sampling period 1995–2006). The total number of specimens (n) by species is indicated. See Table 2 for full species names

Site	Ab	Ap	Aa	An	Ag	Au	Br	Bb	Bj	Ca	Cp	Cn	Cg	Ci	Cc	El	Gg	Gc
n =	197	33	5128	116	117	267	171	224	392	31	166	233	74	24	98	216	1261	15
1	0.012					0.004	0.004	0.004	0.008								0.184	
2		0.128					0.06	0.052				0.036					0.324	
3			0.092				0.032	0.112									0.132	
4	0.008		0.016		0.004					0.028								
5		0.004						0.056									0.068	
6	0.001			0.007						0.001						0.003		
7			0.003	0.001	0.001	0.001			0.001	0.001					0.001	0.001		
8	0.012					0.004							0.016					
9			0.002		0.005		0.005					0.002	0.013			0.001		0.002
10					0.003												0.02	
11	0.002		0.003					0.001	0.003	0.001	0.009	0.001				0.002	0.005	
12	0.012		0.346			0.008		0.012	0.026			0.008				0.01	0.008	
13	0.005		0.255			0.002		0.008	0.023			0.003			0.001		0.055	
14	0.008		0.066		0.001	0.004									0.001	0.005		
15			0.13	0.002	0.003				0.27			0.012				0.003		
16			0.08						0.013							0.008		
17	0.004		0.166					0.006	0.002			0.002				0.01		
18			0.015						0.003								0.017	
19			0.001			0.001				0.002						0.001	0.001	
20			0.061													0.003		
21	0.005		0.318	0.008		0.008		0.008	0.103						0.003		0.045	
22			0.005			0.003			0.002									
23							0.02	0.004									0.068	
24																	0.016	
25			0.012				0.008	0.092				0.016	0.04				0.044	0.016
26					0.004		0.04	0.004				0.016	0.008		0.008		0.148	
27					0.004		0.04										0.008	
28							0.008										0.032	
29			0.036		0.012			0.108				0.076				0.004	0.012	
30			0.02														0.08	0.016
31							0.028									0.032	0.076	

Site	Lc	Li	Ll	Lt	Pf	Pp	Pm	Pr	Rs	Rr	St	Se	Sg	Sl	Th	Tt	Vv
n =	2704	1152	806	20	729	165	44	160	58	2768	166	161	82	52	86	75	15
1	0.056	0.008			0.064			0.056	0.004	0.12		0.004					
2	0.152		0.052					0.008		0.012	0.008					0.004	
3	0.04		0.032		0.004					0.06	0.008						
4	0.004	0.008		0.012	0.012		0.176		0.004	0.036							
5	0.092									0.024	0.148						
6	0.022									0.063		0.005					
7	0.002	0.001								0.002		0.001	0.001			0.001	
8			0.008	0.016						0.012	0.004				0.004		
9	0.032		0.008		0.0167					0.015							
10	0.095																
11	0.008	0.002	0.001		0.002					0.005				0.001			
12	0.108	0.014		0.006	0.006					0.342						0.004	
13	0.06	0.002			0.001					0.145			0.001			0.002	
14	0.011				0.001					0.106		0.028				0.004	
15	0.068	0.003			0.007					0.207		0.002	0.007			0.002	
16										0.249		0.036	0.004			0.009	
17	0.132	0.004								0.154		0.006	0.014			0.002	
18	0.213	0.002	0.002						0.007	0.039		0.013					
19	0.003	0.001								0.001							
20	0.109	0.003							0.001	0.0597		0.011	0.001				
21	0.048	0.008	0.003							0.133		0.003	0.003				
22	0.09				0.002				0.002	0.023		0.003					
23	0.028		0.004		0.188					0.164	0.02				0.008		
24	0.032		0.016		0.012					0.12	0.012				0.012		
25	0.076		0.064		0.004	0.036				0.016					0.004		
26	0.092		0.08		0.012	0.052				0.024	0.012				0.012		
27	0.108										0.012				0.08		
28	0.248		0.004			0.016				0.056	0.004				0.012		
29	0.104		0.036	0.016	0.068					0.032							
30	0.016	0.016			0.016					0.188							0.012
31	0.028		0.028		0.004										0.004		

Table 3
(continued)

Site	Ab	Ap	Aa	An	Ag	Au	Br	Bb	Bj	Ca	Cp	Cn	Cg	Ci	Cc	El	Gg	Gc
n =	197	33	5128	116	117	267	171	224	392	31	166	233	74	24	98	216	1261	15
32													0.084					
33							0.004	0.004								0.004	0.18	
34					0.004		0.004	0.004								0.004	0.308	
35							0.012									0.004	0.376	
36							0.028									0.004	0.456	
37									0.004						0.004	0.004	0.168	
38							0.012										0.128	
39							0.016						0.04					
40													0.008					
41													0.004				0.024	
42							0.016						0.004			0.004		
43					0.004		0.008	0.016									0.124	
44							0.168										0.044	
45							0.004										0.028	0.012
46					0.004			0.008							0.008		0.008	
47							0.092										0.46	
48							0.008										0.24	
49							0.008						0.012				0.008	
50							0.008						0.004					
51	0.001		0.014			0.001		0.002	0.001	0.001		0.001			0.001		0.001	
52			0.008		0.003	0.001						0.001				0.001	0.001	
53			0.027			0.001		0.001				0.001			0.001		0.001	
54	0.001		0.001		0.001	0.001		0.001	0.001			0.001			0.001	0.001		0.001
55	0.002		0.007		0.001	0.001			0.002					0.001		0.001		
56*							0.001						0.001				0.005	
57*	0.002		0.014			0.001		0.002	0.002		0.002	0.001		0.001	0.002	0.001	0.001	
58*			0.002			0.001					0.001					0.001	0.001	
59*	0.003			0.001	0.001				0.001	0.001	0.001				0.001	0.002		
60*			0.003	0.002	0.002	0.001			0.001		0.002				0.001	0.001		
61*			0.007		0.001	0.002						0.001				0.001	0.001	
62*			0.027	0.001		0.001		0.001							0.001		0.001	

Site	Lc	Li	Ll	Lt	Pf	Pp	Pm	Pr	Rs	Rr	St	Se	Sg	Sl	Th	Tt	Vv
n =	2704	1152	806	20	729	165	44	160	58	2768	166	161	82	52	86	75	15
32											0.12						
33	0.016				0.012					0.176							
34	0.064		0.004		0.016					0.22							
35	0.168		0.012					0.028		0.04							
36	0.252	0.012	0.036		0.028					0.128							
37	0.048		0.004		0.048					0.06				0.004			
38	0.096		0.044							0.116							
39						0.26					0.016				0.044		
40						0.004					0.052				0.016		
41	0.144		0.016			0.076				0.016	0.008				0.004		
42						0.012											
43	0.016		0.004							0.008							
44											0.044				0.004		
45	0.02			0.024	0.032	0.052				0.02	0.016				0.004		
46	0.052		0.036							0.132							
47	0.172		0.092						0.076	0.044							
48	0.004		0.008						0.028								
49	0.004					0.016				0.012	0.036				0.072		
50										0.216	0.008				0.02		
51	0.004									0.002			0.001	0.001			0.001
52	0.004	0.007	0.004							0.005				0.001			
53	0.003	0.002	0.003							0.001							
54	0.008		0.002		0.004					0.004				0.001		0.001	
55	0.001	0.002	0.001							0.006			0.002				
56*					0.027	0.002				0.017	0.002				0.001		
57*	0.004									0.003			0.002	0.001			0.001
58*	0.003	0.001						0.002		0.002							
59*	0.002				0.004			0.002		0.001		0.002				0.001	
60*	0.003	0.001							0.001	0.002		0.001	0.001			0.001	
61*	0.004	0.007	0.003							0.005				0.001			
62*	0.003	0.002	0.003							0.001							

Table 4
Species-specific density (ind m^{-2}) of young-of-the-year (YOY) fishes at sampling sites. Long-term sampling sites indicated with asterisks (abundances at these sites are considered for the whole sampling period 1995–2006). The total number of specimens (n) by species is indicated

Site	Ab	Ap	Aa	An	Au	Br	Bb	Bj	Ca	Cn	Cg	El	Ga	Gg	Lc	Li	Ll	Lt
n =	94	4	1920	1280	83	425	358	328	7	51	531	19	7	2009	1570	574	726	18
1			0.032		0.008					0.012				0.172	0.132	0.06		
2		0.004				0.06	0.016							0.148	0.164		0.176	
3			0.012				0.024			0.004				0.076	0.224		0.008	
4			0.012		0.004		0.024								0.048	0.02		
5		0.012					0.104								0.096			
6	0.044					0.092	0.056	0.004	0.012					0.028	0.06			
7			0.088					0.04							0.008		0.004	
8												0.016						0.072
9			0.192											0.132	0.148	0.02	0.024	
10														0.0067				
11			0.272			0.004	0.052			0.112		0.016		1.184	0.076	0.012	0.008	
12			0.004				0.008							0.02	0.004			
13			0.06									0.012		0.024	0.22			
14	0.004												0.007					
15												0.004						
16	0.008		2.772		0.012							0.004	0.02	0.004	0.008			
17	0.188		1.316		0.004			0.088				0.008			0.204			
18			0.216									0.008						
19			0.092															
20			0.012												0.008			
21			0.372									0.004			0.028			
22							0.02								0.008			
23			0.064				0.28								0.064		0.044	
24																		
25						0.004	0.136							0.024	0.072			
26						0.04								0.068	0.264		0.024	
27						0.004								0.108	0.228		0.012	
28			0.008				0.212			0.004					0.02		0.028	
29			0.028				0.008			0.008					0.116	0.004	0.036	
30						0.004								0.024	0.208		0.108	
31											0.012							

Site	Pf	Pp	Pm	Pr	Rs	Rr	St	Se	Sg	Sl	Th	Tt	Vv
n =	244	450	21	21	1406	1353	44	97	2	13	26	5	6
1	0.084				0.08	0.38							
2					0.008	0.004						0.004	
3					0.004	0.036							
4			0.084		0.008	0.012			0.004	0.036			
5							0.012						
6					0.26	0.036		0.036					
7					0.096								
8						2.4							
9				0.004		0.14		0.004					
10													
11					0.004	0.192							
12	0.024					0.02							
13													
14								0.004					
15								0.032	0.004				
16					0.036	0.08							
17					2.312	0.02							
18					1.712								
19					0.02								
20													
21					0.172								
22	0.008					0.004							
23		0.004				0.008							
24	0.12												
25		0.04											
26													
27						0.036							
28	0.004					0.004							
29	0.016					0.024							0.008
30						0.036							
31							0.028						

Table 4
(continued)

Site n =	Ab 94	Ap 4	Aa 1920	An 1280	Au 83	Br 425	Bb 358	Bj 328	Ca 7	Cn 51	Cg 531	El 19	Ga 7	Gg 2009	Lc 1570	Li 574	Ll 726	Lt 18
32			0.028				0.008							0.46	0.028			
33			0.02					0.016		0.004				1.68	0.312		0.212	
34						0.004								0.08	0.288		0.016	
35								0.004						0.008	0.088		0.068	
36			0.004					0.016						0.004				
37						0.004								0.024	0.036			
38						0.004					0.004							
39						0.008	0.004							0.032	0.056		0.168	
40					0.02	0.004	0.016							0.028	0.156		0.008	
41			0.008		0.008	0.004	0.024							0.068	0.084		0.004	
42						0.032								0.072				
43			0.004											0.008	0.036			
44															0.072			
45						0.004								0.164	0.008			
46												0.004		0.04				
47						0.008					0.004							
48						0.004												
49																		
50						0.004												
51			0.028				0.008							0.18	0.016		0.004	
52							0.076			0.004				0.032	0.112	0.088	0.124	
53							0.236			0.004				0.072	0.08	0.028	0.212	
54	0.012		0.064											0.004	0.02			
55			0.08			0.004	0.036							0.036	0.056			
56*						0.06					0.95							
57*			0.32				0.035			0.107				1.392	1.034	0.25	0.075	
58*			0.381												0.156			
59*	0.078		0.121		0.011			0.673						0.235	0.15	0.231	0.022	
60*			0.106	0.961									0.001	0.013	0.109			
61*			0.027		0.019		0.025		0.006		0.002			0.726	0.337	0.579		
62*			0.526		0.248	0.029	0.063							0.021	0.043		1.827	

Site n =	Pf 244	Pp 450	Pm 21	Pr 21	Rs 1406	Rr 1353	St 44	Se 97	Sg 2	Sl 13	Th 26	Tt 5	Vv 6
32					0.02								
33				0.028									
34						0.004							
35	0.032					0.248							
36						0.012							
37													
38													
39		0.14				0.012					0.004		
40		0.004				0.316							
41						0.508							
42													
43	0.064	0.012											
44						0.04		0.02					
45					0.076								
46													
47		0.004					0.02				0.052		
48	0.244						0.008				0.016		
49	0.12												
50	0.244						0.008				0.016		
51													
52						0.084							
53						0.036							
54						0.004		0.004					
55						0.052							
56*		0.89					0.002				0.001		
57*													0.027
58*					0.685								
59*	0.017			0.035	0.053	0.128							
60*					0.006	0.025		0.055					
61*						0.102				0.002			
62*						0.06						0.013	

Table 5
Type 3 tests of fixed effects for final GLMM models

Effect	Num DF	Den DF	F	P <
For GLMM I				
EFI YOY	1	41.1	13.48	0.0007
For GLMM II				
Method	1	109	5.93	0.0165
For GLMM IV				
Time interval	3	75.3	19.96	0.0001

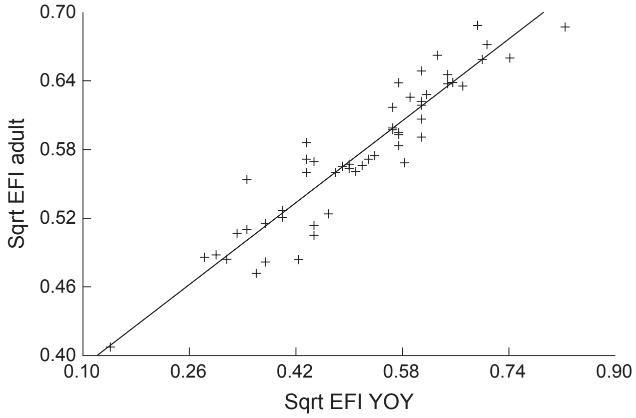

Fig. 2. Relationship between European Fish Index (EFI) assessed with adult and young-of-the-year (YOY) assemblages (predicted values of square root transformed data). Curve fitted by: $y = 0.4469x - 0.3459$; ($n = 55$, $r^2 = 0.88$)

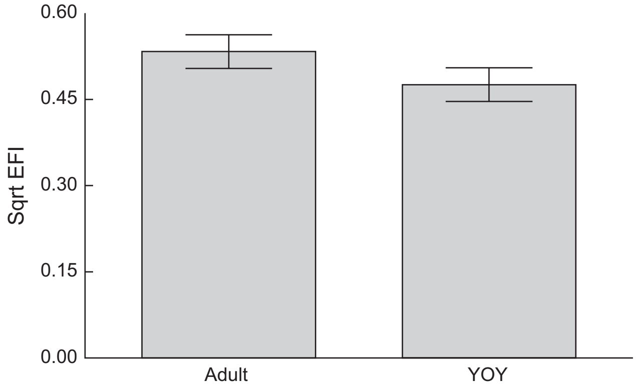

Fig. 3. European Fish Index (EFI) values assessed with adult and young-of-the-year (YOY) sampling methods. Values are adjusted means ± SE of square root transformed data

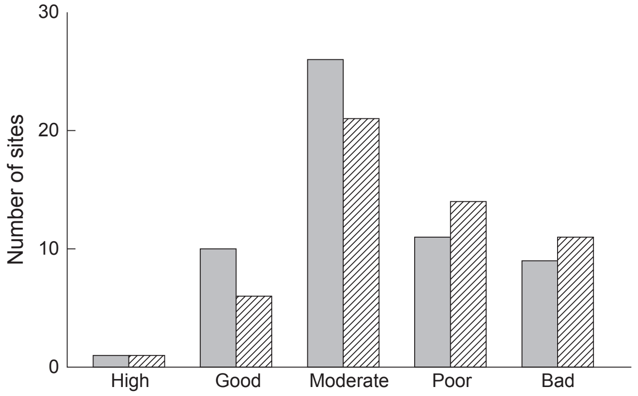

Fig. 4. Number of sites in particular quality classes according to the European Fish Index (EFI) assessed with adult (shaded bar) and young-of-the-year (YOY; striped bar) assemblages (n = 55; years 2003–2007)

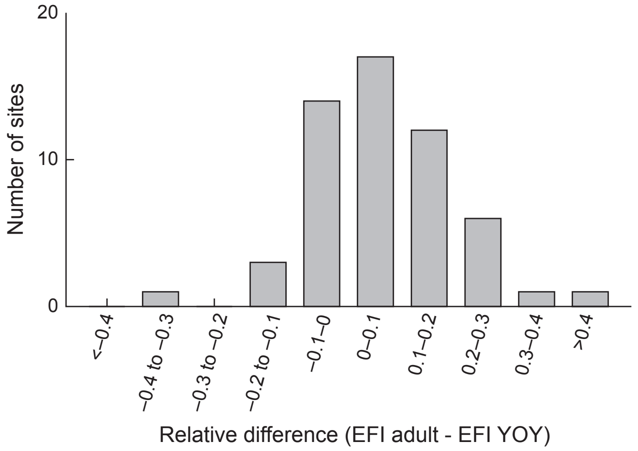

Fig. 5. Histogram of relative differences between European Fish Index (EFI) assessed with adult and young-of-the-year (YOY) assemblages at particular sites (n = 55; years 2003–2007)

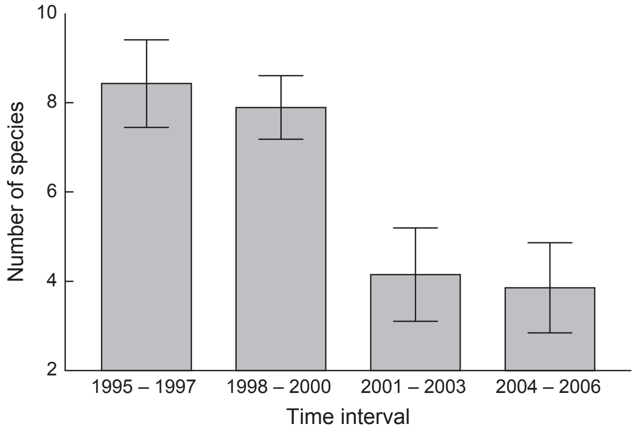

Fig. 6. Number of successfully reproducing species across time intervals (n = 84; 12 years of sampling) in the Elbe River. Values are adjusted means ± SE

indicator of the appropriate age structure of a population, reflecting its vitality and development.

From a practical point of view, samplings of YOY have lower personal and logistic requirements than adult samplings. Especially in medium and large rivers where three persons with basic equipment for sampling of YOY are sufficient, four or more persons, boat(s) and other specific equipment for sampling of adults are needed [requirements depending on the river width–see CEN directive (CEN, 2003) for details]. Time effectiveness is also an issue, considering that multiple numbers of sites per day can be sampled using the YOY methodology. Time consumption of YOY laboratory identification is more or less comparable to the identification of adults, provided that the determining person is experienced and that sampling is conducted in the late summer because of the developing YOY. All above-mentioned aspects indicate sampling of YOY as a more cost-effective procedure than the sampling of adults.

To conclude, YOY sampling represents a useful method for assessing ecological quality of a river, with the ability to provide a sensitive response to the habitat structure. Moreover, the response is relatively fast and reliable, regardless of the longevity of the adult fish assemblages or of stocking. Also, sampling efficiency in the longitudinal gradient is of negligible importance, since YOY gather in shallow areas near the shoreline (Schlosser, 1987) where they can be easily sampled regardless of the river size. To further explore the utility of the YOY assessment, precise analyses of its variability in time and responses to particular pressures are needed.

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References

Balon, E. K., 1984: Reflections on some decisive events in the early life of fishes. Trans. Am. Fish. Soc. **113**, 178–185.

Cattanéo, F., 2005: Does hydrology constrain the structure of fish assemblages in French streams? Local scale analysis. Arch. Hydrobiol. **164**, 345–365.

Cattanéo, F.; Carrel, G.; Lamouroux, N.; Breil, P., 2001: Relationship between hydrology and cyprinid reproductive success in the Lower Rhône at Montélimar, France. Arch. Hydrobiol. **151**, 427–450.

Cattanéo, F.; Lamouroux, N.; Breil, P.; Capra, H., 2002: The influence of hydrological and biotic processes on brown trout population dynamics. Can. J. Fish. Aquat. Sci. **59**, 11–22.

CEN document, 2003: Water quality – Sampling of fish with electricity. CEN/TC 230, Ref. No. EN 14011:2003 E, pp.16.

Copp, G. H., 1989a: Electrofishing for fish larvae and 0 + juveniles: equipment modifications for increased efficiency with short fishes. Aquacult. Fish. Mgmt. **20**, 453–462.

Copp, G. H., 1989b: The habitat diversity and fish reproductive function of floodplain ecosystems. Environ. Biol. Fish. **26**, 1–26.

Copp, G. H., 1990: Effect of regulation on the fish recruitment in the Great Ouse, a lowland river. Regul. Riv. **5**, 251–263.

Copp, G. H., 1992: An empirical model for predicting the microhabitat of 0 + juveniles in lowland streams. Oecologia **91**, 338–345.

Copp, G. H.; Mann, R. H. K., 1993: Comparative growth and diet of tench *Tinca tinca* (L.) larvae and juveniles in river floodplain biotopes in France and England. Ecol. Freshw. Fish **2**, 58–66.

FAME Consortium, 2004: Manual for the application of the European Fish Index - EFI. A fish-based method to assess the ecological status of European rivers in support of the Water Framework Directive. Version 1.1.

Freeman, M. C.; Bowen, Z. H.; Bovee, K. D.; Irwin, E. R., 2001: Flow and habitat effects on juvenile fish abundance in natural and altered flow regimes. Ecol. Appl. **11**, 179–190.

Garner, P., 1995: Suitability indices for juveniles 0 + roach (*Rutilus rutilus*, L.) using point abundance sampling data. Regul. Rivers: Res. Mgmt. **10**, 99–104.

Garner, P., 1996: Diel patterns in the feeding and habitat use of 0-group fishes in a regulated river: The River Great Ouse, England. Ecol. Freshw. Fish **5**, 175–182.

Houde, E. D., 1994: Differences between marine and freshwater fish larvae: implications for recruitment – ICES (International Council for the Exploration of the Sea). J. Mar. Sci. **51**, 91–97.

Janáč, M.; Jurajda, P., 2007: A comparison of point abundance and continuous sampling by electrofishing for age-0 fish in a channelized lowland river. N. Am. J. Fish. Manage. **27**, 1119–1125.

Kenward, M. G.; Roger, J. H., 1997: Small sample inference for fixed effects from restricted maximum likelihood. Biometrics **53**, 983–997.

Kryzhanovsky, S. G., 1949: Eco-morphological principles and patterns of development among minnows, loaches and catfishes. Part II: ecological groups of fishes and patterns of their distribution. Akad. Nauk SSSR, Tr. Inst. Morf. Zhitovr im Akad. AN Severtsova **1**: 237–231. (Fish. Res. Board Can. Transl. Series No. 2945, 1974).

Lightfoot, G. W.; Jones, N. V., 1996: The relationship between the size of 0-group roach, (*Rutilus rutilus* (L.)), their swimming capabilities, and their distribution in an English river. Folia Zool. **45**, 355–360.

Maitland, P. S.; Lyle, A. A., 1991: Conservation of freshwater fish in the British Isles: the current status and biology of threatened species. Aquat. Conserv. **1**, 23–54.

Oberdorff, T.; Hughes, R., 1991: Modification of an Index of Biotic Integrity based on fish assemblages to characterize rivers of the Seine Basin, France. Hydrobiologia **228**, 117–130.

Oberdorff, T.; Guilbert, E.; Luccheta, J. C., 1993: Patterns of fish species richness in the Seine River basin, France. Hydrobiologia **259**, 157–167.

Pianka, E. R., 1970: On r- and K- selection. Am. Nat. **104**, 592–597.

Pont, D.; Allardi, J.; Belliard, J.; Boët, P.; Carrel, G.; Changeux, T.; Oberdorff, T.; Olivier, J. M.; Persat, H.; Poizat, G., 1995: Life-history traits of the fish fauna of French rivers: first results. Bull. Fr. Pêche Piscic. **337/338/339**, 113–119.

Schiemer, F.; Keckeis, H.; Kamler, E., 2003: The early life history stages of riverine fish: ecophysiological and environmental bottlenecks. Comp. Biochem. Physiol. Part A **133**, 439–449.

Schlosser, I. J., 1985: Flow regime, juvenile abundance, and the assemblage structure of stream fishes. Ecology **66**, 1484–1490.

Schlosser, I. J., 1987: The role of predation in age- and size-related habitat use by stream fishes. Ecology **68**, 654-659.

Schmutz, S.; Cowx, I. G.; Haidvogl, G.; Pont, D., 2007: Fish-based methods for assessing European running waters: a synthesis. Fish. Manage. Ecol. **14**, 369–380.

Tao, J.; Little, R.; Patetta, M.; Truxillo, C.; Wolfinger, R., 2002: Mixed SAS System Course Notes. SAS Institute Inc., Cary.

Winemiller, K. O.; Rose, K. A., 1992: Patterns of life-history diversification in North American fishes: implications for population regulation. Can. J. Fish. Aquat. Sci. **49**, 2196–2218.

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CHAPTER V

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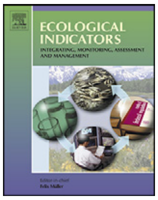

The response of the young of the year fish to river obstacles: Functional and numerical linkages between dams, weirs, fish habitat guilds and biotic integrity across large spatial scale

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ABSTRACT

River obstacles are recognised as being among the serious ecological concerns, having negative effects on biodiversity and ecosystem functions. They have been shown to affect fish community structure, which represents a fundamental component of the biological integrity of riverine ecosystems. However, a general quantitative relationship that links the response of fish community structure to river obstacles across larger spatial scales is lacking. We assessed young of the year (YOY) fish (27,596 specimens and 35 species) from a total of 54 study sites distributed across three international river basins. The YOY fish community structure was expressed as the probability of habitat-related guild dominance. We also examined the indicative value and responsiveness of two large spatial-scale, fish-based indices of biological integrity (IBI), the European Fish Index (EFI) and the Czech multi-metric index (CZI). We analysed two types of obstacles (1118 weirs and 28 dams for a total of 2126 obstacles) for which numerical and distance-related descriptors were derived using a geographic information system (GIS). All the obstacles considered together were found to be the most relevant predictor of YOY fish community structure response. The negative impact on YOY fish (a loss of rheophilic species and low IBIs values) increased with the increasing number of obstacles and the decreasing relative distance between two consecutive obstacles, indicating the habitat quality and availability and the poor connectivity of particular watersheds. This investigation allowed us to identify the functional and numerical responses of the YOY fish community structure to river obstacles across hydrological networks. Our results contribute an important prerequisite to river restoration efforts, identifying where in the hydrological networks either ecological studies or restoration actions would produce the most effective results.

1. Introduction

River obstacles and dams in particular, are recognised as serious ecological challenges (Nilsson et al., 2005). They interrupt ecological connectivity and functional pathways of matter, energy and organisms, and they modify the structures and functions of ecosystems (Ward and Standford, 1995; Postel et al., 1996) to thereby affect all of components of riverine ecosystems (Pringle, 2001; Freeman et al., 2003). Among the aquatic biota affected by obstacles, fish have received considerable scientific attention. The negative impacts include species extinction and decline (Duncan and Lockwood, 2001), limited dispersal (Fukushima et al., 2007), declining population persistence, diminished growth and fitness (Morita and Yamamoto, 2001), genetic differentiation and variation (Dunham and Rieman, 1999) and changes in fish community structure (Freeman and Marcinek, 2006).

The literature concerning fish communities within regulated rivers comprises both comparative and experimental studies that have related the fish community response (Bunn and Arthington, 2002; Poff and Zimmerman, 2010) to one or more types of alterations caused by river obstacles (i.e., impoundments, flow regulation, fragmentation) on local (Pusey et al., 1993; Freeman et al., 2001) or regional scales (Poff and Allan, 1995; Freeman and Marcinek, 2006; Taylor et al., 2008). However, increasing number of studies have emphasised that studying and managing riverine fish involves the use of an appropriate spatial dimension (Schlosser, 1991; Fausch et al., 2002; Poizat and Pont, 1996). Similarly, very few studies have quantified the response of fish communities to varying degrees of alterations per se. Freeman and Marcinek (2006) as well as Poff and Zimmerman (2010) have demonstrated that fish communities consistently show negative responses to

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Abbreviations: IBI, index of biotic integrity; YOY, young of the year; EFI, European Fish Index; CZI, Czech multi-metric index; EQR, ecological-quality ratio; T_S, typical species presence; *A*, abundance; R_S, relative abundance of rheophilic species; E_S, relative abundance of eurytopic species; GIS, geographical information system; GLMM, general linear mixed models; AIC, Akaike's information criterion; GEE, generalised estimating equation; CR, Czech Republic.

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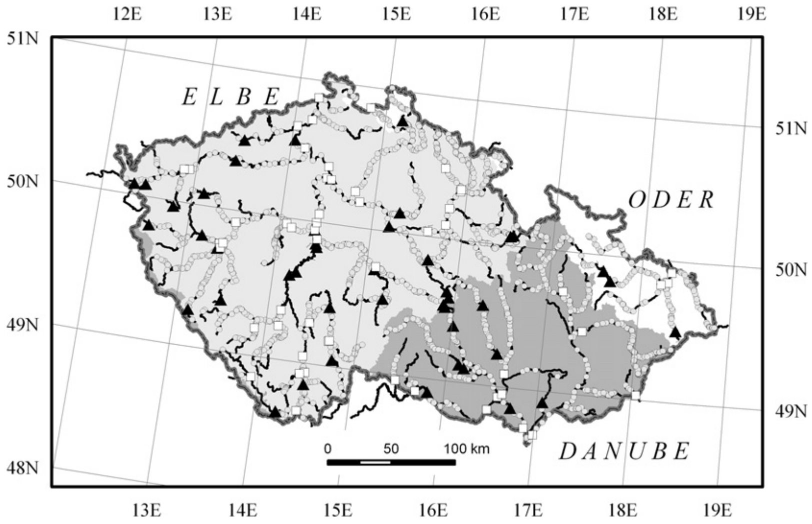

Fig. 1. Map of the Czech Republic showing three hydrological networks with the locations of dams (▲), weirs (○), and sampling sites (□) analysed in this study.

hydrological alterations. Other types of alterations associated with obstacles such as fragmentation, are also significant (Fukushima et al., 2007). With these considerations in mind, it is logical to hypothesise that the response of fish community structure to the multiple alterations caused by obstacles is related to their spatial distribution. A great deal of research has focused on analysing the effects of single or multiple dams on adult and juvenile fish (e.g. Freeman et al., 2001; Clavero et al., 2004; Santucci et al., 2005). However, less attention has been paid to the impacts of small obstacles, for which only a few studies on adult fish have been published (Tiemann et al., 2004; Gillette et al., 2005; Chick et al., 2006; Poulet, 2007; Beatty et al., 2009).

The index of biotic integrity (IBI) is a tool that originates from the predictable and quantifiable response of fish communities to human alterations on aquatic ecosystems. Based on the original IBI concept (Karr, 1981), other related IBIs were subsequently developed. IBI principles were also used during the implementation of the Water Framework Directive 2000/60/ECC (WFD) within the European Union (EU). The WFD requires the classification of ecological quality for all inland waters, and fish are used as one of the biological quality elements. Fish are typically assessed using a unified IBI, the European Fish Index (EFI), as well as other related national methods (Schmutz et al., 2007). One important prerequisite for the use of these methods is an appropriate level of IBI responsiveness to human pressures (Angradi et al., 2009), but the use of such a methodology has yet to be published regarding river obstacles.

Therefore, in this article, we attempt to identify the quantitative relationships between river obstacles and fish community structure to either support or reject the hypothesis that fish communities will show both functional and numerical responses to obstacles at a river scale across the hydrological network of the Czech Republic (CR). We chose to focus on juvenile fish (young of the year, YOY) because multiple alterations associated with obstacles have been shown to affect the reproductive success and juvenile survival of riverine fish populations (Schlosser, 1985; Copp et al., 1991). Reproductive success and juvenile survival are major drivers of recruitment and thus limit the viability of populations. The larger scale of our study is of particular importance, as it links biotic processes in rivers at scales that are relevant to the human alterations made to watersheds (Schlosser, 1991; Fausch et al., 2002). Individual rivers vary with respect to the degree of fragmentation relative to their size. River obstacles also vary by type and thus differ in their direct and indirect environmental impacts (Poff and Hart, 2002). Therefore, we have investigated the effects of the number, type and spatial placement of obstacles relative to the scale of the river. To analyse fish community structure independently of natural longitudinal zonation (Sheldon, 1968; Vannote et al., 1980), we used the habitat-based functional-guild dominance approach (Oberdorff et al., 2001, 2002; Pont et al., 2006) considering fish species-specific habitat requirements (rheophillic species, all freshwater life stages linked to lotic environment; eurytopic species, tolerant to either lotic or lentic environments, its dominance in riverine ecosystems would be indicative of alteration), as well as two recently developed multi-metric IBIs, the EFI (Pont et al., 2006; Schmutz et al., 2007) and the national Czech multi-metric index (CZI) (Horký and Slavík, 2009). An additional goal of this study was to verify the responsiveness of each IBI to river obstacles.

2. Methods

2.1. Study area

We examined data from a total of 54 study sites, including rivers ranging in size from small to large (distance from the source ranged from 40 to 377 km) and those that are either pristine or greatly affected by human activities. All of the study sites were selected as representative sites for particular river stretches. The following variables were taken into account: river morphology, hydrology, geomorphological type, channelization, impoundment, local habitat alteration and point sources of pollution. Information about these variables was gathered from special maps, local authorities and field surveys. All of the study sites were distributed across three international river basins (the Elbe River, the Danube River, and the Oder River) within the CR hydrological network. At each site, surveys were carried out over two subsequent years (2006 and 2007) (Fig. 1). Other surveys were excluded to limit bias related to the analysis of YOY fish community structure over time.

2.2. Sampling procedures

Fish were collected using electrofishing equipment (Honda GXV 50, 225–300 V, 6 A, pulsed DC) adopted for YOY sampling (Jurajda et al., 2010) by wading along the bank in an upstream direction regardless of the size of the river. A stratified sampling procedure, which covered all habitat types, was applied to obtain a representative sample of the site. Although point abundance and continuous sampling of YOY fish are comparable in terms of the resulting qualitative analyses, continuous sampling was preferred to permit a quantitative interpretation of the results (Janáč and Jurajda, 2007). Most fish were identified by species and immediately released at the site of capture. Specimens that could not be reliably identified were fixed in a 4% formaldehyde solution for subsequent laboratory analysis. The sampling area borders were estimated using a portable GPS receiver (GPS map 76S, Garmin Ltd., USA). All surveys were undertaken during the late summer (August to mid-September) to ensure efficient sampling of the YOY fish (Copp et al., 1991; Jurajda et al., 2010).

The location (coordinates) and type of each obstacle were extracted from the DIBAVOD T.G. Masaryk Water Research Institute database (www.dibavod.cz). The data were verified using topographic maps and aerial photographs. With regard to river obstacles in the longitudinal profile, this database serves as the most comprehensive information source at the national level. However, these data had certain limitations; only dams larger than 5×10^4 m^2 and located in the main river channel and weirs higher than 0.5 m could be used for further spatial analyses (Fig. 1).

2.3. Data analysis

All fish species were assigned to habitat-related ecological (habitat) guilds, according to the methods of Schiemer and Waidbacher (1992). A habitat guild was defined as dominant if its relative site-specific abundance (number of specimens belonging to a particular ecological guild divided by the total number of specimens) was greater than 50%. The EFI values for each site were computed using the EFI software (EU FAME, 2004). The EFI reflects the ecological status of the site and ranges from 0 to 1 to indicate poor to high ecological status, respectively (Pont et al., 2006; Schmutz et al., 2007). Correspondingly, the CZI was also computed for each site. At altitudes lower than 800 m (which is the case for all sites evaluated in this study), the CZI consists of four metrics that are expressed as an ecological-quality ratio (EQR) between the observed and expected values for a particular river type. These four metrics include the EQR of the typical species presence (T_S), the EQR of the overall abundance (A), the EQR of the relative abundance of rheophilic species (R_S) and the EQR of the relative abundance of eurytopic species (E_S). This index, which consists of values ranging from 0 to 1, is expressed as following formula:

$$\mathrm{CZI} = \frac{(T_S + A + R_S) - (E_S)}{4}$$

The locations of the study sites and river obstacles were entered into the geographical information system (GIS) and projected using ArcGIS and Arc Macro Language software to create vector and raster layers. The derived descriptors (obstacle type, number of obstacles, number of obstacles upstream and downstream, and the distance between two consecutive obstacles) were quantified using the ArcGIS distance functions of the ARC module. The descriptors were derived for each river and expressed as relative values (%) of the observed descriptor and river length. The descriptors were considered according to two obstacle types, which resulted in the following three categories: dams, weirs and all of the river obstacles combined.

2.4. Statistical analysis

All of the data were analysed with the aid of the SAS statistical package (version 9.1; SAS Institute Inc.) and were appropriately transformed as necessary to meet normality and homoscedasticity requirements. Three complex general linear mixed models (GLMM) were applied for each dependent variable (GLMM I to III for EFI and GLMM IV to VI for CZI). GLMM is a generalisation of the standard linear model used in the GLM procedure, where the generalisation is that the data are permitted to exhibit correlation and non-constant variability (Breslow and Clayton, 1993; Tao et al., 2002). The mixed linear model therefore provides flexibility for modelling not only the means of the data (as in the standard linear model) but their variances and covariances as well. The variances of the random-effects parameters, commonly known as variance components, become the covariance parameters for the particular model. To account for repeated measurements at the same sites, the analyses were performed with site codes and with site codes nested within years as random factors using PROC MIXED.

For the GLMM models, the fixed effects were identical in terms of the following continuous variables: the total number of obstacles, total number of dams, total number of weirs, distance between two consecutive obstacles and the distance between the two closest obstacles in relation to river length. Thus, the variables for GLMM I and IV were derived from all river obstacles, the variables for GLMM II and V were derived from weirs and the variables for GLMM III and VI were derived from dams.

The significance of each fixed effect (including interactions) in the mixed GLMM was assessed using an *F*-tests, where the least-significant effect, beginning with a full model, was sequentially dropped (backward selection procedure) (Tao et al., 2002). Fixed effects that were not statistically significant were not discussed further. Associations between the dependent variable and other continuous variables were estimated by fitting a random-coefficient model using PROC MIXED, as described by Tao et al. (2002). Using this random-coefficient model, predicted values were calculated for the dependent variable and plotted against the continuous variable. The degrees of freedom were calculated using the Kenward–Roger method (Kenward and Roger, 1997).

Akaike's information criterion (AIC) was used to select the best predictor among the three categories of obstacles. A second-order AIC for small sample sizes (AICc) was used, and models with smaller AICc values were preferred (Anderson et al., 2000). The variables derived from the selected predictor were used for the following GENMOD analyses.

The GENMOD procedure for binomial distributions was designed to estimate the probability that a particular ecological group would be dominant. We applied an analysis of categorical, repeated measurements based on the generalised estimating equation (GEE) approach (Liang and Zeger, 1986). The GEE is an extension of generalised linear models and provides a semi-parametric approach to longitudinal data analysis. Separate models were applied for each habitat guild. The explanatory variables tested were identical with the variables used in the GLMM analyses. For each analysis, a full model containing all of the explanatory variables and interactions was initially fitted. Each term was then excluded from the full model unless doing so had a significant effect on the fit of the model. Only statistically significant variables were discussed further. To account for repeated measures at the same sites, we used a REPEATED statement with the interaction of the site code and the year defined in the SUBJECT option. The GEE analysis produces score statistics, parameter estimates with standard errors, confidence intervals, *Z* and Wald statistics and *P*-values. The *Z* and Wald statistics generally produce more liberal *P*-values than score statistics do. Stokes et al. (2000) recommended reporting the more conservative score statistics for a Type 3 GEE analysis, particularly for those with small sample sizes. Therefore, we refer only to the score statistics in this study.

3. Results

Data from a total of 33 rivers were included in the analyses. Based on the GIS, a total of 1118 weirs and 28 dams were located

Table 1
Type 3 tests of fixed effects for final GLMM models (Num DF – numerator degrees of freedom; Den DF – denumerator degrees of freedom; AICc – Akaike's information criterion for small sample sizes). Only GLMM I and IV models are considered further and their results are expressed in Fig. 2.

Effect	Num DF	Den DF	*F*	*P*	AICc
GLMM I – EFI – all river obstacles					
No. of obstacles × distance	1	54	8.85	0.01	−120.2
GLMM II – EFI – weirs					
No. of obstacles × distance	1	54	8.61	0.01	−119.1
GLMM III – EFI – dams					
No. of obstacles × distance	1	54	3.99	0.05	−115.6
GLMM IV – CZI – all river obstacles					
No. of obstacles × distance	1	54	5.65	0.02	−289.7
GLMM V – CZI – weirs					
No. of obstacles × distance	1	54	5.30	0.03	−288.4
GLMM VI – CZI – dams					
No. of obstacles × distance	1	54	3.74	0.05	−280.6

in the study area, and the numbers of weirs and dams in particular rivers ranged from 4 to 81 (upstream, 0–81; downstream, 0–46) and from 0 to 5 (upstream, 0–5; downstream, 0–5), respectively. The distance between each study site and two consecutive dams varied from 16.4 to 209.3 km (upstream, 13.7–197.5 km; downstream, 1.1–128.4 km). The distance between each study site and two consecutive weirs as well as the distance between both obstacle types combined ranged from 0.7 to 42.9 km (upstream, 0.3–23.4 km; downstream, 0.4–19.5 km), corresponding to 0.3–28.3% of the particular river length (refer to Fig. 1 and Supplementary material, Appendix A).

A total of 27,596 YOY fish belonging to 35 species were caught. All samplings included fish, and all study sites contained several species (4–16, with median value per site of 10). A total relative site-specific abundance ranged from 110 to 2618 individuals, and the length of the sampled study sites ranged from 110 to 279 m. Among the most dominant rheophilic fish, the species frequently encountered (frequency of occurrence ≥30%) included *Squalius cephalus* (1.0–69.9% of total species abundance), *Gobio gobio* (0.3–79.0%), *Barbus barbus* (0.8–30.2%), *Leuciscus leuciscus* (0.1–40.1%), *Barbatula barbatula* (0.2–44.8%), *Chondrostoma nasus* (0.1–11.1%), *Aspius aspius* (0.1–1.2%) and *Leuciscus idus* (0.2–70.7%).

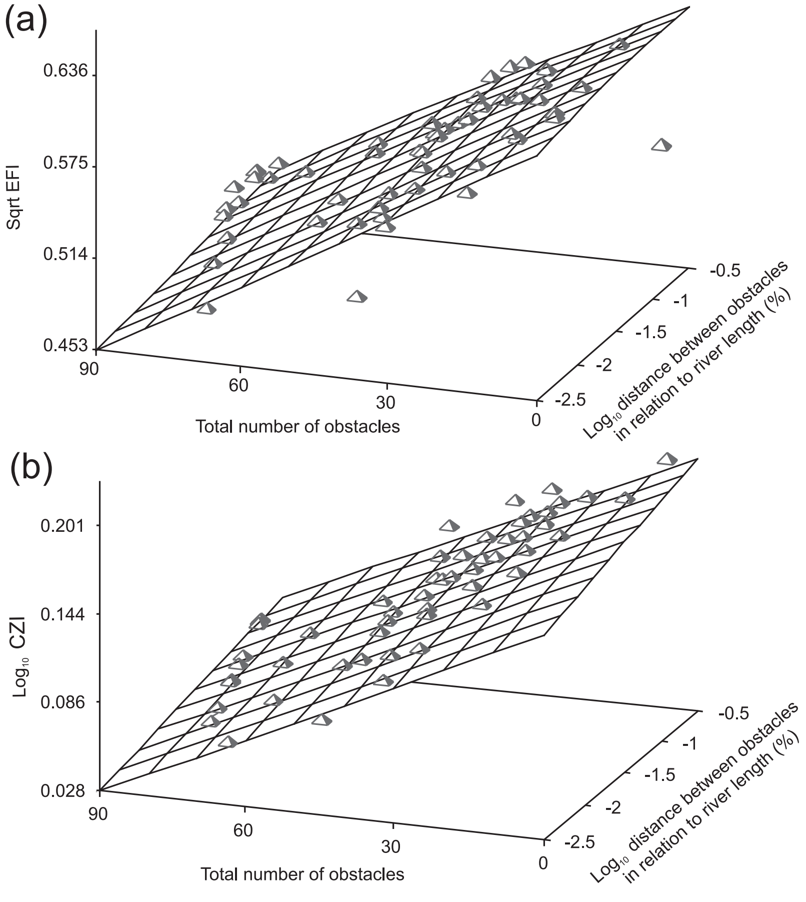

Fig. 2. Predicted values of (a) square-root-transformed European Fish Index (EFI) and (b) log10-transformed Czech multi-metric index (CZI) plotted against the total number of obstacles according to the distance between the obstacles in relation to the length of the river.

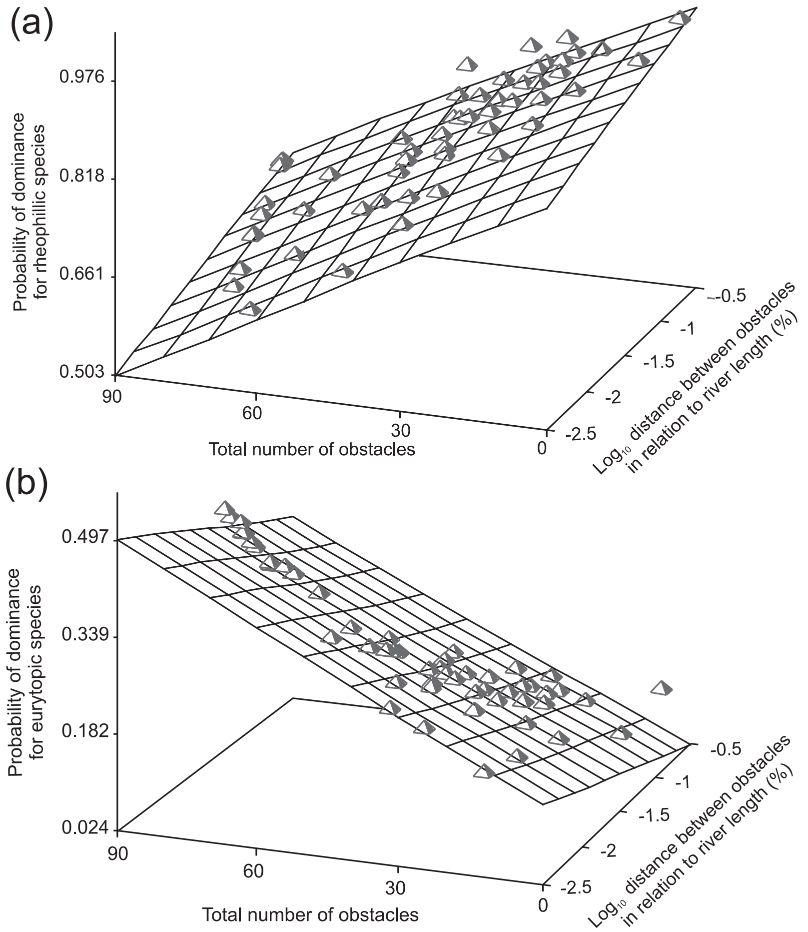

Fig. 3. Probability of dominance for (a) rheophilic and (b) eurytopic fish species in relation to the total number of obstacles according to the distance between the obstacles in relation to the length of the river.

The most frequently recorded eurytopic species were *Alburnus alburnus* (0.1–49.7%), *Rutilus rutilus* (0.2–84.6%), *Perca fluviatilis* (0.1–87.5%), *Pseudorasbora parva* (0.1–15.4%) and *Abramis brama* (0.1–8.1%). Limnophilic species were *Scardinius erythrophthalmus* (0.1–6.7%), *Tinca tinca* (0.4%) and *Carassius carassius* (1.5%) and these were recorded only occasionally. The estimated IBI values ranged from 0 to 0.61 (EFI, median, 0.32) and from 0 to 0.83 (CZI, median, 0.41), corresponding to bad and good ecological statuses, respectively (refer to Supplementary material, Appendices A and B).

According to the AIC model selection criteria, all of the obstacles combined were found to represent the most relevant predictor of the responsiveness of the YOY fish community structure, as expressed by the EFI and CZI values (Table 1). Thus, only models that included all of the obstacles were further considered. The EFI and CZI values showed similar trends (Fig. 2a and b); these values decreased with increasing numbers of obstacles in a particular river and with decreasing relative distances between two consecutive obstacles with respect to the length of the river. In other words, greater numbers of obstacles and shorter distances between the obstacles were associated with more degraded fish community structures at a particular site. This result was underscored by low probability of the dominance of rheophilic species ($\chi^2 = 5.34$, d.f. = 1, $P < 0.02$; Fig. 3a) and the correspondingly a high probability of dominance ($\chi^2 = 5.34$, d.f. = 1, $P < 0.02$; Fig. 3b) of eurytopic species at such sites.

4. Discussion

Most previous studies have focused on the effects of single or multiple obstacles and have typically been site-based to evaluate obstacles (McLaughlin et al., 2006; Poulet, 2007; Navarro-Llácer et al., 2010) or cumulative effects (Santucci et al., 2005) at local and/or regional scales. Most studies involving larger individual-river or river-segment scales have reported a general and consistent response of fish communities and/or biotic integrity throughout the studied area (Santucci et al., 2005). In contrast, few studies have demonstrated the response of fish communities to the degree of alterations associated with river obstacles (Freeman and Marcinek, 2006; Taylor et al., 2008; Poff and Zimmerman, 2010). In this study, we observed clear functional and numerical responses of YOY fish to obstacles at a river scale across the hydrological network of the CR. All of the variables used to characterise YOY fish community structure (i.e., dominance of particular ecological guilds and IBI indices) were strongly related to the spatial distribution of the obstacles. Thus, our results are consistent with the hypothesis that fish community structure responds to river obstacles in relation to the degree of the alteration caused by the obstacles.

Based on our analyses, the obstacles were of strong ecological significance, irrespective of their type (i.e., dam or weir). Although small obstacles, such as weirs, have less dramatic ecological effects than dams (Poff and Hart, 2002), their numbers were more than two orders of magnitude greater in our study area. Weirs are numerous within river networks, but their cumulative effects are rarely considered. Like dams, they modify local habitats, influence the environmental suitability of particular species and contribute to changes in adult (Tiemann et al., 2004; Gillette et al., 2005; Poulet, 2007) and YOY fish community structure.

In this study, the negative impact on YOY fish community structure (i.e., the loss of rheophilic species dominance and low IBI values) increased with decreasing relative distances between consecutive obstacles and their increasing numbers. Given the variety of known habitat-related alterations associated with river obstacles (Baxter, 1977; Ward and Standford, 1995), increasing their numbers and decreasing the relative distances between them likely affects the overall quality and the proportion of degraded habitat areas in watersheds (Poff and Hart, 2002; Paukert et al., 2011). This was clearly evidenced by the observed patterns of both IBIs. In addition to environmental changes, the serious impact caused by river obstacles is a “barrier effect”, disrupting or limiting the migration of organisms throughout the river system (Pringle, 2001; Fagan, 2002; Freeman et al., 2003). The barrier effect (i.e., longitudinal connectivity) may also be expected to vary as a function of obstacle number and mutual distance (Lucas and Baras, 2001; Cote et al., 2009).

Our results complement many previous studies in which rheophilic fish were shown to exhibit a greater detrimental response to the alterations associated with river obstacles compared to eurytopic species, as shown by adult (e.g., Penzcak et al., 1998; Quinn and Kwak, 2003; Santucci et al., 2005; Freeman and Marcinek, 2006; McLaughlin et al., 2006; Taylor et al., 2008) and juvenile fish (Copp et al., 1991; Freeman et al., 2001). Rheophilic fish display complex habitat requirements (Persat et al., 1994; Schiemer et al., 2003) and often perform long-distance movement behaviours to complete their life cycles (Lucas and Baras, 2001). Habitat-related alterations associated with river obstacles induce a loss of structural complexity (habitat homogenisation), which reduces the quality, size and spatial distribution of vital habitats that are required for different life stages and activities, such as spawning and/or nursing. Therefore, the lack of habitat availability and the poor connectivity of watersheds likely act as multiple environmental bottlenecks that lead to population declines. This is exemplified by the conservation status of rheophilic species worldwide (Duncan and Lockwood, 2001). In contrast, unspecialised eurytopic fish benefit from structurally novel habitats, as they often have limited migratory behaviours and further contribute to the inability of rheophilic fish to maintain viable populations through competition for food and space or through predation (Beatty et al., 2009).

An important criterion for effective river restoration is that ecological improvement should be measurable (Palmer et al., 2005). Our approach provided a means of quantifying the negative impacts of river obstacles over larger river scales and across hydrological networks. This approach is conceptually simple but implies important methodological and practical challenges. First, obstacles must be mapped and analysed using high-spatial-resolution GIS tools. Second, the use of YOY fish instead of adults provides several methodological advantages (Jurajda et al., 2010) and greater indicative values for complex environmental alterations (Copp et al., 1991; Freeman et al., 2001; Schiemer et al., 2003). Third, fish are highly sensitive organisms that respond significantly to many types of alterations (Karr, 1981; Fausch et al., 1990; Bunn and Arthington, 2002). In this study, fish community structure was expressed as the probability of the dominance of particular fish habitat guilds and by two IBIs, the EFI and the CZI. The EFI is an approved, standardised fish-based methods of bio-assessment within the EU WFD. However, this index was previously not calibrated with real pressure/obstacle data (Pont et al., 2006; Schmutz et al., 2007). In this context, the high responsiveness of both IBIs is particularly important and supports their use as effective tools for bio-assessment and for the prioritisation, evaluation and planning of river-restoration actions. Our approach, however, must be interpreted with caution. The observed quantitative relationships included many types of multiple interacting alterations that are associated with obstacles, and we would thus expect to find complex effects on YOY fish community structure. Given the scale of our study, however, we cannot address each of these alterations, although they all act to affect the responses of fish communities (Bunn and Arthington, 2002; Poff and Zimmerman, 2010). Similarly, we accounted for general aspects of fish community structure (i.e., the dominance of particular habitat-related guilds) rather than examining species composition or richness to describe the large dataset. This may have resulted in some degree of uncertainty. For instance, the derived quantitative relationships are fully adequate and applicable for the conservation management of rheophilic fish displaying potamodromous life histories with regular upstream and downstream movements (Lucas and Baras, 2001). However, this is not true for diadromous species, whose distribution and dispersal are spatially linked with their access to upstream areas (Fukushima et al., 2007).

5. Conclusions

Because demands on water resources are likely to create more obstacles in the future (Nilsson et al., 2005), there is an urgent need to understand the nature of river obstacles for identifying and prioritising management issues. In this context, our approach will not replace ecological studies documenting the sensitivity of particular fish populations, species or communities to river obstacles, including assessments of environmental factors. On the contrary, all of these activities are necessary for understanding the drivers of the present conditions, which environmental factors should be improved, and how river-restoration efforts should proceed. Our approach, however, permits the identification of the functional and numerical responses of YOY fish community structure to river obstacles across hydrological networks. We observed strong quantitative relationships between the spatial distribution of obstacles and YOY fish community structure, indicating the habitat quality, availability and the connectivity of particular watersheds. We also demonstrated the ecological significance of all obstacle types, the adequate indicative value of YOY fish and the high level of responsiveness of both IBIs (EFI, CZI). Our results contribute an important prerequisite for planning river-restoration efforts, identifying where in hydrological networks ecological studies or restoration actions would produce the most effective results. Therefore, our approach should assist with the prioritisation of future restoration actions and will support water resource-management decision making for the conservation of rheophilic fish communities and the improvement of biotic integrity in fragmented river ecosystems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2012.05.018.

References

Anderson, D.R., Burnham, K.P., Thompson, W.L., 2000. Null hypothesis testing: problems, prevalence and an alternative. J. Wildlife Manage. 64 (4), 912–923.

Angradi, T.R., Pearson, M.S., Jicha, T.M., Taylor, D.L., Bolgrien, D.W., Moffett, M.F., Blocksom, K.A., Hill, B.H., 2009. Using stressor gradients to determine reference expectations for great river fish assemblages. Ecol. Indic. 9, 748–764.

Baxter, R.M., 1977. Environmental effects of dams and impoundments. Annu. Rev. Ecol. Syst. 8, 255–283.

Beatty, R.J., Rahel, F.J., Hubert, W.A., 2009. Complex influences of low-head dams and artificial wetlands on fishes in a Colorado River tributary system. Fish. Manage. Ecol. 16, 457–467.

Breslow, N.E., Clayton, D.G., 1993. Approximate inference in generalized linear mixed models. J. Am. Stat. Assoc. 88, 9–25.

Bunn, S.E., Arthington, A.H., 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environ. Manage. 30, 492–507.

Chick, J.H., Pegg, M.A., Koel, T.M., 2006. Spatial patterns of fish communities in the upper Mississippi river system: assessing fragmentation by low-head dams. River Res. Appl. 22, 413–427.

Clavero, M., Blanco-Garrido, F., Prenda, J., 2004. Fish fauna in Iberian Mediterranean river basins: biodiversity, introduced species and damming impacts. Aquat. Conserv. 14, 575–585.

Copp, G.H., Oliver, J.M., Peňáz, M., Roux, A.L., 1991. Juvenile fishes as functional describers of fluvial ecosystem dynamics: applications on the Rhone, France. Regul. River 6, 135–145.

Cote, D., Kehler, D.G., Bourne, C., Wiersma, Y.F., 2009. A new measure of longitudinal connectivity for stream networks. Landscape Ecol. 24, 101–113.

Duncan, J.R., Lockwood, J.L., 2001. Extinction in a field of bullets: a search for causes in the decline of the world's freshwater fishes. Biol. Conserv. 102, 97–105.

Dunham, J.B., Rieman, B.E., 1999. Metapopulation structure of bull trout: influences of physical, biotic, and geometrical landscape characteristics. Ecol. Appl. 9, 642–655.

EU FAME (Fish-based Assessment Method for the Ecological Status of European Rivers), 2004. Manual for the Application of the European Fish Index – EFI. A Fish-based Method to Assess the Ecological Status of European Rivers in Support of the Water Framework Directive. Version 1.1. Available from: http://fame.boku.ac.at/downloads (accessed on 20.04.10).

Fagan, W.F., 2002. Connectivity, fragmentation, and extinction risk in dendritic metapopulations. Ecology 83, 3243–3249.

Fausch, K.D., Lyons, J., Karr, J.R., Angermeier, P.L., 1990. Fish communities as indicators of environmental degradation. Am. Fish. Soc. Symp. 8, 123–144.

Fausch, K.D., Torgersen, C.E., Baxter, C.V., Li, H.W., 2002. Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. BioScience 52 (6), 483–498.

Freeman, M.C., Marcinek, P.A., 2006. Fish assemblage responses to water withdrawals and water supply reservoirs in Piedmont streams. Environ. Manage. 38 (3), 435–450.

Freeman, M.C., Bowen, Z.H., Bovee, K.D., Irwin, E.R., 2001. Flow and habitat effects on juvenile fish abundance in natural and altered flow regimes. Ecol. Appl. 11 (1), 179–190.

Freeman, M.C., Pringle, C.M., Greathouse, E.A., Freeman, B.J., 2003. Ecosystem-level consequences of migratory faunal depletion caused by dams. Am. Fish. Soc. Symp. 35, 255–266.

Fukushima, M., Kameyama, S., Kaneko, M., Nakao, K., Steel, E.A., 2007. Modelling the effects of dams on freshwater fish distributions in Hokkaido, Japan. Freshwater Biol. 52, 1511–1524.

Gillette, D.P., Tiemann, J.S., Edds, D.R., Wildhaber, M.L., 2005. Spatiotemporal patterns of fish assemblage structure in a river impounded by low-head dams. Copeia 2005, 539–549.

Horký, P., Slavík, O., 2009. Czech national method of the river ecological status classification according to the fish biocoenosis. VÚV TGM, v.v.i. Report, Prague, 31 pp.

Janáč, M., Jurajda, P., 2007. A comparison of point abundance and continuous sampling by electrofishing for age-0 fish in a channelized lowland river. N. Am. J. Fish. Manage. 27, 1119–1125.

Jurajda, P., Slavík, O., White, S., Adámek, Z., 2010. Young-of-the-year fish assemblages as an alternative to adult fish monitoring for ecological quality evaluation of running waters. Hydrobiologia 644, 89–101.

Karr, J.R., 1981. Assessment of biotic integrity using fish communities. Fisheries 6 (6), 21–27.

Kenward, M.G., Roger, J.H., 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53, 983–997.

Liang, K.Y., Zeger, S.L., 1986. Longitudinal data analysis using generalized linear models. Biometrika 73 (1), 13–22.

Lucas, M.C., Baras, E., 2001. Migration of Freshwater Fishes. Blackwell Science, Oxford.

McLaughlin, R.L., Porto, L., Noakes, D.L.G., Baylis, J.R., Carl, L.M., Dodd, H.R., Goldstein, J.D., Hayes, D.B., Randall, R.G., 2006. Effects of low-head barriers on stream fishes: taxonomic affiliations and morphological correlates of sensitive species. Can. J. Fish. Aquat. Sci. 63, 766–779.

Morita, K., Yamamoto, S., 2001. Effects of habitat fragmentation by damming on the persistence of stream-dwelling charr populations. Conserv. Biol. 16, 1318–1323.

Navarro-Llácer, C., Baeza, D., De las Heras, J., 2010. Assessment of regulated rivers with indices based on macroinvertebrates, fish and riparian forest in the southeast of Spain. Ecol. Indic. 10, 935–942.

Nilsson, Ch., Reidy, C., Dynesius, M., Revenga, C., 2005. Fragmentation and flow regulation of the world's large river systems. Science 308, 405–408.

Oberdorff, T., Pont, D., Hugueny, B., Chessel, D., 2001. A probabilistic model characterizing fish assemblages of French rivers: a framework for environmental assessment. Freshwater Biol. 46, 399–415.

Oberdorff, T., Pont, D., Hugueny, B., Porcher, J.P., 2002. Development and validation of a fish-based index (FBI) for assessment of river health in France. Freshwater Biol. 47, 1720–1734.

Palmer, M.A., Bernhardt, E.S., Allan, J.D., Lake, P.S., Alexander, G., Brooks, S., Carr, J., Clayton, S., Dahm, C.N., Follstad, S.J., Galat, D.L., Loss, S.G., Goodwin, P., Hart, B., Hassett, D.D., Jenkinson, R., Kondolf, G.M., Lave, R., Meyer, J.L., O'Donnell, T.K., Pagano, L., Sudduth, E., 2005. Standards for ecologically successful river restoration. J. Appl. Ecol. 42, 208–217.

Paukert, C.P., Pitts, K.L., Whittier, J.B., Olden, J.D., 2011. Development and assessment of a landscape-scale ecological threat index for the Lower Colorado River Basin. Ecol. Indic. 11, 304–310.

Penzcak, T., Glowacki, L., Galicka, W., Koszalinski, H., 1998. A long-term study (1985–1995) of fish populations in the impounded Warta River, Poland. Hydrobiologia 368, 157–173.

Persat, H., Olivier, J.M., Pont, D., 1994. Theoretical habitat templets, species traits, and species richness: fish in the Upper Rhone River and its floodplain. Freshwater Biol. 31, 439–454.

Poff, N.L., Allan, J.D., 1995. Functional organization of stream fish assemblages in relation to hydrological variability. Ecology 76, 606–627.

Poff, N.L., Hart, D.D., 2002. How dams vary and why it matters for the emerging science of dam removal. BioScience 52, 659–668.

Poff, N.L., Zimmerman, J.K.H., 2010. Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. Freshwater Biol. 55, 194–205.

Poizat, G., Pont, D., 1996. Multi-scale approach to species–habitat relationships: juvenile fish in a large river section. Freshwater Biol. 36, 611–622.

Pont, D., Hugueny, B., Beier, U., Goffaux, D., Melcher, A., Noble, R., Rogers, C., Roset, N., Schmutz, S., 2006. Assessing river biotic condition at a continental scale: a European approach using functional metrics and fish assemblages. J. Appl. Ecol. 43, 70–80.

Postel, S.L., Daily, G.C., Ehrlich, P.R., 1996. Human appropriation of renewable freshwater. Science 271, 785–788.

Poulet, N., 2007. Impact of weirs on fish communities in a Piedmont stream. River Res. Appl. 23, 1038–1047.

Pringle, C.M., 2001. Hydrological connectivity and the management of biological reserves: a global perspective. Ecol. Appl. 11, 981–998.

Pusey, B.J., Arthington, A.H., Read, M.G., 1993. Spatial and temporal variation in fish assemblage structure in the Mary River, south-east Queensland: the influence of habitat structure. Environ. Biol. Fish. 37, 355–380.

Quinn, J.W., Kwak, T.J., 2003. Fish assemblage changes in an Ozark river after impoundment: a long-term perspective. Trans. Am. Fish. Soc. 132, 110–119.

Santucci, V.J., Gephard, S.R., Pescitelli, S.M., 2005. Effects of multiple low-head dams on fish, macroinvertebrates, habitat, and water quality in the Fox River, Illinois. N. Am. J. Fish. Manage. 25, 975–992.

Schiemer, F., Waidbacher, H., 1992. Strategies for conservation of a Danubian fish fauna. In: Boon, P.J., Calow, P., Petts, G.E. (Eds.), River Conservation and Management. Wiley, New York, pp. 363–382.

Schiemer, F., Keckeis, H., Kamler, E., 2003. The early life history stages of riverine fish: ecophysiological and environmental bottlenecks. Comp. Biochem. Phys. A 133, 439–449.

Schlosser, I.J., 1985. Flow regime, juvenile abundance, and the assemblage structure of stream fishes. Ecology 66, 1484–1490.

Schlosser, I.J., 1991. Stream fish ecology: a landscape perspective. BioScience 41, 704–712.

Schmutz, S., Cowx, I.G., Haidvogl, G., Pont, D., 2007. Fish-based methods for assessing European running waters: a synthesis. Fish. Manage. Ecol. 14, 369–380.

Sheldon, A.L., 1968. Species diversity and longitudinal succession in stream fishes. Ecology 49, 193–198.

Stokes, M.E., Davis, C.S., Koch, G.G., 2000. Categorical Data Analysis Using the SAS System, 2nd ed. SAS Institute Inc., Cary, NC.

Tao, J., Littell, R., Patetta, M., Truxillo, C., Wolfinger, R., 2002. Mixed Model Analyses Using the SAS System Course Notes. SAS Institute Inc., Cary, NC.

Taylor, C.M., Millican, D.S., Roberts, M.E., Slack, W.T., 2008. Long-term change to fish assemblages and the flow regime in a southeastern U.S. river system after extensive aquatic ecosystem fragmentation. Ecography 31, 787–797.

Tiemann, J.S., Gillette, D.P., Wildhaber, M.L., Edds, D.R., 2004. Effects of lowhead dams on riffle-dwelling fishes and macroinvertebrates in a midwestern river. T. Am. Fish. Soc. 133, 705–717.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., Cushing, C.E., 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37 (1), 130–137.

Ward, J.V., Standford, J.A., 1995. Ecological connectivity in alluvial river ecosystems and its disruption by flow regulation. Regul. River 11, 105–119.

APPENDIX

Horká, P., Ibbotson, A., Jones, J. I., Cove, R. J., Scott, L. J. (2010).

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Validation of scale-age determination in European grayling *Thymallus thymallus* using tag-recapture analysis

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To validate age determination from scales in European grayling *Thymallus thymallus*, the scale-read age of fish was compared with the true age obtained by tag-recapture analysis. A total of 3997 individuals were tagged with visible implant tags and passive integrated transponder (PIT) tags in the River Wylye, south-west England during 1999–2007. Annual repeat surveys were undertaken and collected scales read without prior knowledge of tag-recapture age. Accuracy of fish ageing by scales was highest in 1 and 2 year-old fish but decreased in older fish. In later life stages (>4 years old), underestimation of age occurred and the error in reading scales rose to 51·9% in 5 year-old fish. Age assigned from scales underestimated the tag-recapture assigned age by as much as 3 years. This study suggests that use of scales is an appropriate method to age a short-lived population of *T. thymallus* inhabiting productive lotic systems. The underestimation of age in older fish, however, needs to be considered in the management of fish stocks because it may lead to undesirable exploitation of population.

Key words: annuli; fish ageing; longevity; salmonids; tagging; Thymallidae.

INTRODUCTION

Estimates of age are fundamental to an understanding of the biology of fishes and the dynamics of fish populations (Beamish & McFarlane, 1983). Age determination methods have received considerable attention as they provide the basic data used to assess the structure, growth and mortality of fish populations. Often such information is of crucial importance for fisheries management decisions, especially in fresh waters (Mills & Beamish, 1980). Methods used to determine the age of fishes need to be validated and errors minimized wherever possible (Britton *et al.*, 2004), as incorrect

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estimates of age may lead to a misunderstanding of the biology and population dynamics of species (Beamish & McFarlane, 1983). The most widely used methods of age determination use the structure of scales or otoliths (Morales-Nín *et al*., 1998; Maceina & Sammons, 2006). The number of annuli formed on scales serve as an accurate method for ageing many species of freshwater fishes, especially species whose scales have easily recognized annuli, such as cyprinids (Hellawell, 1974; Mann, 1976; Mann & Steinmetz, 1985) and salmonids (Nordeng, 1961; Hellawell, 1969). Accuracy in this technique of age determination, however, varies among species (Beamish & Fournier, 1981; Chang, 1982; Kimura & Lyons, 1991), suggesting the validity of a method should be established for each species of fish studied (Beamish & McFarlane, 1983).

Literature on the growth and age of European grayling *Thymallus thymallus* (L.) is scarce. Descriptions of the growth patterns of scales were provided by Jones (1953) and Hellawell (1969), who noted annual changes in scale morphology and the occurrence of discrete annuli corresponding with age groups. The accuracy of scale ageing in *T. thymallus*, however, has not been verified. Such verification is often lacking as it requires independent estimates of age, usually made on individual fish over long periods of time. Tag-recapture analysis provides a useful growth comparison for quantifying the accuracy of ageing when well-sampled age classes are used (Campana, 2001). The objective of the study is to provide a validation of accuracy of scale ageing in *T. thymallus* which was not available in previous studies. To achieve this objective, long-term mark-recapture data on *T. thymallus* from the River Wylye, south-west England were collected in eight consecutive years.

MATERIALS AND METHODS

Samples of scales were collected from the *T. thymallus* caught in the River Wylye (51° 10′ 59″ N; 2° 6′ 30″ W), a spring-fed chalk stream draining into the River Avon. The fish were collected using semi-quantitative electrofishing each October for eight consecutive years (1999–2007). Using long-term mark–recapture data, the real ages of fish of all age categories were provided. In total, 3997 individually tagged *T. thymallus* were examined. Fish were tagged with VI tags (North-West Marine Technology; www.nm-inc.com) and passive integrated transponder (PIT) tags (Trovan, Ltd; www.trovan.com). In total, 1579 fish were tagged using PIT tags and 2418 fish were tagged with VI tags. In both cases, the fish were anaesthetized with 2-phenoxy ethanol (0·2 ml l^{-1}). The VI tag was inserted into the clear flesh behind the left eye using a VI tag injector. The PIT tag was inserted into the body cavity after making a small incision in the body wall. The fish were sampled for scales at tagging and then again at recapture. The fork length (L_F) of each fish was measured to the nearest mm. Scales were removed from the pectoral region above the lateral line and stored in envelopes for subsequent examination. Scales were placed between two microscope slides, magnified and viewed with a Projectina projecting microscope (www.projectina.ch). Two to five scales were used from each fish. For each scale, the lateral radius of the dorsal part of the scale was measured. Regenerated or distorted scales were not used. The scales had to be discarded in 8% of the fish sampled due to distortion or scarring of the scales. Scales from all sampled fish were viewed to confirm ages. All scale readings were made without prior knowledge of the age determined by tagging or of the size of the fish.

The surface of the *T. thymallus* scale is characterized by a corrugated anterior margin and concentric rings, the spacing of which varies markedly and gives a banding effect to the scale. These circuli can be classified into wide summer rings and slender winter rings, set close together [Fig. 1(a)]. The second category is termed a check and is usually readily distinguished. The age can be related to the number of growth checks (annuli) that are related to

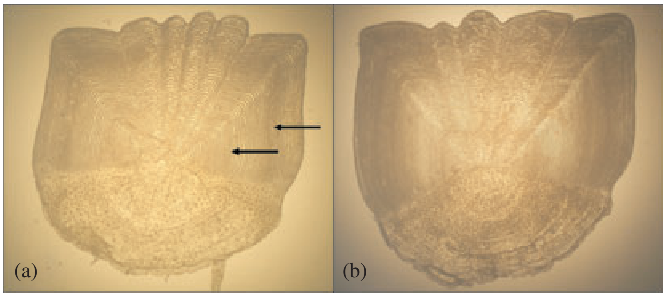

FIG. 1. A scale taken from *Thymallus thymallus* at (a) 290 and (b) 400 mm fork length (L_F) from the River Wylye. (a) Details of readily distinguishable winter and summer rings and corrugated anterior of scale (→, growth checks). (b) The outermost part of scale formed a border with growth checks formed close together.

the elapsed years of life on the assumption that spawning occurs in spring (Hellawell, 1969). These annuli associated with growth checks were used for ageing, following the method of determination used by Hellawell (1969). The validation of the ages was achieved from the tagged 0+ year fish whose age was certain as they formed a separate L_F group within the age classes (Fig. 2), enabling certain separation from older age classes. Ideally, only *T. thymallus* aged 0+ years would have been used as the starting point for all subsequent validations. An insufficient number of fish marked at age 0+ years were recaptured to validate ages in the older age categories. Therefore, most of the *T. thymallus* used in the analysis were tagged at the scale-read age of 1+ years. The validation of 1+ year aged fish demonstrated that scale reading was correct in 97% of cases (Table I). While using 1+ year aged fish would lead to some error in validation of older age groups, this initial error was small and would always overestimate age.

To determine the influence of incorrect age determination caused by scale reading on estimation of the age of *T. thymallus* in the River Wylye population, true age and scale-read age of individuals were compared using regression analyses. To determine the overall influence of inaccuracies in scale-read age, a linear regression model was fitted to the data using a general linear model (GLM) in SAS (www.sas.com). As it was clear that there may have been an age bias in deviation between scale-read age and tag age, a generalized additive model (GAM)

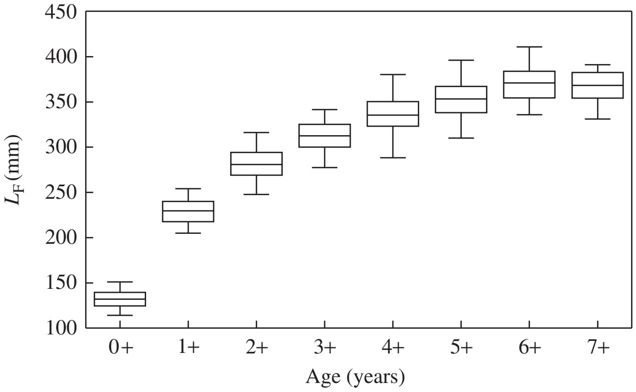

FIG. 2. Fork length (L_F) of age classes (values are mean with range 5 to 95% percentiles, $n = 996$) of marked *Thymallus thymallus* from the River Wylye (age classes were determined by recapture).

TABLE I. The percentage of *Thymallus thymallus* from the River Wylye within real age year classes (determined by tag recapture) that were assigned to scale-read age classes. Values in boldface indicate the percentage of each real age class that were correctly assigned by scale reading

	Scale-read age (% of real age year class)							
	1+	2+	3+	4+	5+	6+	7+	*n*
Real age (years)								
1+	**96·6**	3·4						466
2+	0·8	**95·1**	3·4	0·4	0·4			264
3+		5·1	**86·6**	7·0	0·6		0·6	157
4+		2·9	18·8	**66·7**	8·7	2·9		69
5+			14·8	22·2	**48·1**	3·7	11·1	27
6+				20·0	10·0	**60·0**		10
7+					100·0		**0·0**	3

was fitted to the relationship between tag age and scale age using the mgcv library in R2.8.0. Unlike other regression analyses, such as GLM, this statistical procedure does not presume *a priori* the nature of the function, rather GAM uses smooth link functions, which allow a more flexible specification for the dependence of the response on the covariable. GAM was used to avoid biasing the results by a presumption of deviation between tag age and scale age in certain age classes.

RESULTS

The minimum size of fish was 93 mm L_F and the maximum size 422 mm L_F. The agreement between scale-read age and the age determined by tag-recapture varied among age categories (Table I). The annuli formed on the scales of young fish were easier to read than in older fish, for which the annuli at the edge of the scale were not so clearly visible [Fig. 1(b)]. Agreement with tag-recapture (true) age was highest in 1+ and 2+ year fish, with an error of 4·15%. The percentage of incorrect scale readings increased in older age classes. There was a tendency to underestimate the age of fish aged 4+ years. The age of most fish aged ≥5+ years was incorrectly assigned using scales (Table I). Ages were incorrectly assigned to all fish that were aged 7+ years, the oldest age class encountered in the study (Table I).

Scale-read age was related to true age by the linear equation ($P < 0{\cdot}001$, $n = 996$) and indicated that overall the age of individuals in this population was underestimated by $9{\cdot}2 \pm 2{\cdot}6\%$ (mean ± S.E.) by scale reading (Fig. 3). Although there was no statistical relationship between tag age and the residuals of this line (slope = 0, $P < 0{\cdot}001$), there was clear bias in incorrect scale-read age towards older individuals. Furthermore, data from older age classes (>4+ years) had considerably more leverage, a measure of the effect of the data on the slope of the function, than from younger age classes (<4+ years). Therefore, the relationship between scale age and tag age was explored with GAM. This analysis indicated significant and increasing deviation between scale-read age and tag age for individuals ≥5+ years old (Fig. 3), which suggests that the age of older individuals (and therefore maximum age) cannot be accurately determined from scales.

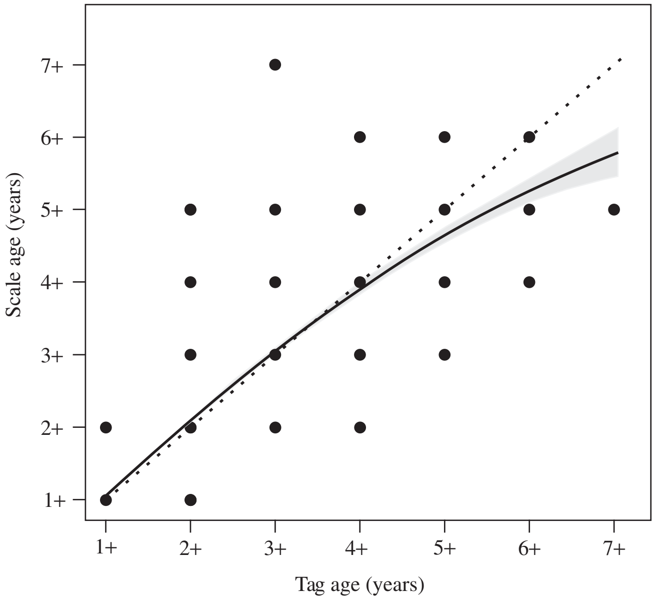

FIG. 3. Relationship between scale-read age and tag (true) age of *Thymallus thymallus* from the River Wylye. The curve was fitted using general additive models in R: $y = 0{\cdot}908\ (\pm 0{\cdot}026)\ x + 0{\cdot}19\ (\pm \text{S.E.})$; shaded area indicates 95% CI of line. The line where scale age = tag age is also shown (........). Due to discrete age classes multiple data points are given (●) ($R^2_{\text{adj}} = 0{\cdot}884$, deviance explained = 88·5%, GCV score = 0·153, $n = 996$, $P < 0{\cdot}001$).

DISCUSSION

Accurate determination of fish ages is essential for fisheries management as it provides an effective tool for assessing growth parameters and the production of fish populations, which in turn are important for fish stock assessment and sustainable exploitation rates. Incorrect estimation of fish age using scales as a method of age determination has been reported in previous studies (McBride *et al*., 2005; Maceina & Sammons, 2006). A lack of accuracy in ageing with scales may occur due to the formation of checks near to the scale margin, particularly in older fishes (Hellawell, 1974). In younger fishes, concentric rings are easily distinguished and the age class of a fish is designated. In older fishes, the annuli at the edge of the scale are more concentrated, often creating a dense edge (structureless edge or edge of closely packed sclerite fragments) with an imprint of corrosion (Nordeng, 1961). Such appearance may be caused by scales tending to grow more slowly and form narrower annuli indicating reduced growth in older fishes (Nordeng, 1961; Carlander, 1974). Scale ageing may also be complicated by the reabsorption or deposition of false annuli, which can appear as a consequence of food limitation or stress (Beamish & McFarlane, 1987; DeVries & Frie, 1996).

The reported maximum ages of *T. thymallus* are highly variable and dependent on geographical location and environmental conditions. Davies *et al*. (2004) reported that most of the *T. thymallus* in southern waters of Britain die by their fifth (4+ years) or sixth year (5+ years), and a further two studies from the Rivers Dove

(T. E. Jacklin, unpubl. data) and Test (Crundwell, 1991) reported the maximum age of *T. thymallus* to be 5 years. The present results suggest that *T. thymallus* in lowland, southern chalk rivers in the U.K. live longer than this and are in more accordance with the results of Woolland (1987), who reported a maximum age of 6–7 years from the more upland River Dee catchment and Llyn Tegid, Wales. The chalk river *T. thymallus* grow extremely fast and the certainty of their 0+ year age further comes from that *T. thymallus* have been reported regularly in the scientific literature as growing to sizes between 100 and 150 mm in their first year (Hellawell, 1969, 1971; Woolland, 1972). At higher latitudes, *T. thymallus* tend to grow more slowly and to live proportionately longer. Fish up to 10 years old have been reported in Scandinavia (Sømme, 1935). Other factors may be involved, such as the maximum age of *T. thymallus* in the former Yugoslavia was reported as 13 years (Woolland, 1987). Based on observations from the former Czechoslovakia, *T. thymallus* has been described as a short-lived species. Of the material examined in seven streams in the drainage area of the Svratka River, only 1·95% of specimens were >5 years (Lusk, 1975), and *T. thymallus* in Bela River only achieve a maximum age of 3 years (Nagy, 1984). Participation in spawning, when a considerable number of individuals perish from fungal or other infectious diseases, is the reason for the apparently short life span of *T. thymallus* (Kupka, 1967). Nevertheless, it is not clear whether any of these estimates of longevity are accurate. The present results suggest that maximum ages based on scales readings are underestimates and that *T. thymallus* live longer than indicated in previous studies.

The present study demonstrated the suitability of scale ageing for productive systems, in which the *T. thymallus* are short-lived and annular marks on fish scales widely spaced. Results of scale ageing, however, should be interpreted with caution in older fish, whose real age is underestimated. The applicability of this method would, however, differ according to geographical location, life conditions, growth rate, life span and species composition of populations. Several studies of northern populations of species closely related to *T. thymallus* revealed that age estimation from scales is not appropriate (Nordeng, 1961; Power, 1978), underestimating the otolith-assigned age by as much as 20 years in Arctic grayling *Thymallus arcticus* (Pallas) (DeCicco & Brown, 2006). This is because otoliths constantly lay down clear annuli during the life of the fish, whereas scale annuli become denser with age (Nordeng, 1961). Also ontogenesis may play an important role, as was shown in the Arctic charr *Salvelinus alpinus* (L.) whose scales ceased to form recognizable growth zones after sexual maturation (Nordeng, 1961). Such results emphasize the need to validate the accuracy in ageing for each species of fishes studied (Beamish & Fournier, 1981).

The perception of the longevity of a species may influence estimates of defining life-history traits such as age at maturity, rate of growth, year-class production and fecundity. Correct estimates of such variables are critical for fish stock assessment and implementation of appropriate exploitation strategies (Power, 1978; Beamish & McFarlane, 1983; Cope & Punt, 2007). Errors in age determination may affect population estimates either through incorrect population growth parameters or in the construction of growth curves, which are not correctly related to the time scale or the cohorts involved (Beamish & McFarlane, 1983). Recognition of distinct age classes or cohorts is an important requirement for the construction of accurate life tables (Le Cren, 1974). The present results suggest that scale-age determination underestimates true age by *c*. 10% and that the rate of survival of the *T. thymallus* population studied is higher than would be predicted from scale-age determination. Hence, the population would have a corresponding lower growth potential than expected from scale readings. Such an underestimate could lead to more exhaustive exploitation of the population on the assumption that renewal of the population would occur at a faster rate. Age is also important in predicting maturity of fish populations (Baum *et al*., 2004). Reproductive effort has been shown to increase with age in several species, as a consequence of increases in both absolute fecundity and egg size with age or size (Mann & Mills, 1979). Egg size influences the size and survival of the larvae (Bagenal, 1969). Mortality has been shown to influence maturation patterns in populations of *T. thymallus* in central Norway (Haugen, 2000). Given that the life span of the species is longer than predicted by scale reading, it is likely that sexual maturity occurs later than previously estimated (Wootton, 1985). Using presumption of earlier maturation based on the incorrect scale-read age, selective fishing in younger age categories could cause a decrease in the number of fish attaining sexual maturity. This may have a detrimental effect on population size as the number of eggs and young fry would be reduced. The imposition of appropriate management strategies including increased size limits to protect juvenile fishes, which are not yet sexually mature, from exploitation. The effect of incorrect ageing on population parameters varies if inaccuracies are biased by the age of fishes. According to Le Cren (1974), unbiased errors in ageing, especially for older fishes, will have little effect on production estimates, as they are few in number and relatively slow growing and, thus, contribute little to the total production. Attention should be paid, however, when estimating production by younger age classes, which often contribute the greater part of production.

The present study has shown that use of scales is an appropriate method for assessing age of short-lived population *T. thymallus* inhabiting productive systems. The accuracy of the method declines with fish age leading to an underestimate of the age of older fish. This inaccuracy should be considered in the formulation of management strategies. This method would probably not be accurate for populations of slower growing *T. thymallus*.

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References

Bagenal, T. B. (1969). Relationship between egg size and fry survival in brown trout *Salmo trutta* (L.). *Journal of Fish Biology* **1,** 349–353.

Baum, D., Laughton, R., Armstrong, J. D. & Metcalfe, N. B. (2004). Altitudinal variation in the relationship between growth and maturation rate in salmon parr. *Journal of Animal Ecology* **73,** 253–260.

Beamish, R. J. & Fournier, D. A. (1981). A method for comparing the precision of a set of age determinations. *Canadian Journal of Fisheries and Aquatic Sciences* **38,** 982–983.

Beamish, R. J. & McFarlane, G. A. (1983). The forgotten requirement for age validation in fisheries biology. *Transactions of the American Fisheries Society* **112,** 735–743.

Beamish, R. J. & McFarlane, G. A. (1987). Current trends in age determination methodology. In *The Age and Growth of Fish* (Summerfelt, R. C. & Hall, G. E., eds), pp. 15–42. Ames, IA: The Iowa State University Press.

Britton, J. R., Cowx, I. G. & Peirson, G. (2004). Sources of error in the ageing of stocked cyprinids. *Fisheries Management and Ecology* **11,** 415–417.

Campana, S. E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology* **59,** 197–242. doi: 10.1006/jfbi.2001.1668

Carlander, K. D. (1974). Difficulties in ageing fish in relation to inland fishery management. In *Ageing of Fish, Proceedings of the International Symposium* (Bagenal, T. B., ed.), pp. 200–205. Old Woking: Unwin Brothers.

Chang, W. Y. B. (1982). A statistical method for evaluating the reproducibility of age determination. *Canadian Journal of Fisheries and Aquatic Sciences* **39,** 1208–1210.

Cope, J. M. & Punt, A. E. (2007). Admitting ageing error when fitting growth curves: an example using the von Bertalanffy growth function with random effects. *Canadian Journal of Fisheries and Aquatic Sciences* **64,** 205–218. doi: 10.1139/F06-179

Crundwell, C. R. (1991). A study of the Grayling, *Thymallus thymallus* on the River Test at Leckford and Longparish, Hampshire, England. Diploma Thesis, Sparsholt College, Hampshire, UK.

Davies, C. E., Shelley, J., Harding, P. T., McLean, I. F. G., Gardiner, R. & Peirson, G. (2004). *Freshwater Fishes in Britain the Species and Their Distribution*. Colchester: Harley Books.

DeCicco, A. L. & Brown, R. J. (2006). Direct validation of annual growth increments on sectioned otoliths from adult Arctic grayling and a comparison of otolith and scale ages. *North American Journal of Fisheries Management* **26,** 580–586.

DeVries, D. R. & Frie, R. V. (1996). Determination of age and growth. In *Fisheries Techniques* (Murphy, B. R. & Willis, D. W., eds), pp. 483–512. Bethesda, MD: American Fisheries Society.

Haugen, T. O. (2000). Growth and survival effects on maturation pattern in populations of grayling with recent common ancestors. *Oikos* **90,** 107–118.

Hellawell, J. M. (1969). Age determination and growth of the grayling *Thymallus thymallus* (L.) of the River Lugg, Herefordshire. *Journal of Fish Biology* **1,** 373–382.

Hellawell, J. M. (1971). The food of the grayling *Thymallus thymallus* (L.) of the River Lugg, Herefordshire. *Journal of Fish Biology* **3,** 187–197.

Hellawell, J. M. (1974). The problems of protracted check formation and the validity of the use of scales in age determination exemplified by two populations of dace *Leuciscus leuciscus* (L.). In *Ageing of Fish, Proceedings of the International Symposium* (Bagenal, T. B., ed.), pp. 173–180. Old Woking: Unwin Brothers.

Jones, J. W. (1953). Age and growth of the trout, grayling, perch and roach of Llyn Tegid (Bala) and the roach of the River Birkett. *Ministry of Agriculture, Fisheries and Food, Fishery Investigations. Series I* **5,** 1–18.

Kimura, D. K. & Lyons, J. J. (1991). Between-reader bias and variability in the age-determination process. *Fishery Bulletin* **89,** 53–60.

Kupka, J. (1967). The applicability of female graylings for repeated spawning. *Bulletin VÚRH Vodňany* **2,** 23–33 (in Czech with English summary).

Le Cren, E. D. (1974). The effects of errors in ageing in production studies. In *Ageing of Fish, Proceedings of the International Symposium* (Bagenal, T. B., ed.), pp. 221–224. Old Woking: Unwin Brothers.

Lusk, S. (1975). Distribution and growth rate of grayling (*Thymallus thymallus*) in the drainage area of the Svratka River. *Zoologicke Listy* **24,** 385–399.

Maceina, M. J. & Sammons, S. M. (2006). An evaluation of different structures to age freshwater fish from a northeastern US rivers. *Fisheries Management and Ecology* **13,** 237–242.

Mann, R. H. K. (1976). Observations on the age, growth, reproduction and food of the chub *Squalius cephalus* (L.) in the River Stour, Dorset. *Journal of Fish Biology* **8,** 265–288.

Mann, R. H. K. & Mills, C. A. (1979). Demographic aspects of fish fecundity. *Symposia of the Zoological Society of London* **44,** 161–177.

Mann, R. H. K. & Steinmetz, B. (1985). On the accuracy of age-determination using scales from rudd, *Scardinius erythrophthalmus* (L.), of known age. *Journal of Fish Biology* **26,** 621–628.

McBride, R. S., Hendricks, M. L. & Olney, J. E. (2005). Testing the validity of Cating's (1953) method for age determination of American shad using scales. *Fisheries* **30,** 10–18.

Mills, K. H. & Beamish, R. J. (1980). Comparison of fin-ray and scale age determinations for lake whitefish (*Coregonus clupeaformis*) and their implications for estimates of growth and annual survival. *Canadian Journal of Fisheries and Aquatic Sciences* **37,** 534–544.

Morales-Nín, B., Torres, G. J., Lombarte, A. & Recasens, L. (1998). Otolith growth and age estimation in the European hake. *Journal of Fish Biology* **53,** 1155–1168.

Nagy, S. (1984). On the age, growth and food of grayling (*Thymallus thymallus* L.) in the River Bela (western Tatra mountains). *Laboratorium Rybarstva a Hydrobiologie Bratislava Czechoslovakia* **4,** 301–326.

Nordeng, H. (1961). On the biology of char (*Salmo alpinus* L.) in Salangen, North Norway 1. Age and spawning frequency determined from scales and otoliths. *Nytt Magasin for Zoologi* **10,** 67–123.

Power, G. (1978). Fish population structure in arctic lakes. *Journal of the Fisheries Research Board of Canada* **35,** 53–59.

Sømme, S. (1935). Verst og naering hos harr og ørret (*Thymallus thymallus* L. og *Salmo trutta* L.). *Nytt Magasin for Naturidenskapene* **75,** 187–218.

Woolland, J. V. (1972). Studies on salmonid fishes in Llyn Tegid and the Welsh Dee. PhD Thesis, University of Liverpool, Liverpool, UK.

Woolland, J. V. (1987). Grayling in the Welsh Dee Part 3, Feeding. *Journal of the Grayling Society* **Spring,** 15–20.

Wootton, R. J. (1985). Energetics of reproduction. In *Fish Energetics* (Tyler, P. & Calow, P., eds), pp. 231–254. Baltimore, MD: Johns Hopkins University Press.