This manuscript is contextually identical with the following published paper:
Tatár, S., Bajomi, B., Specziár, A., Tóth, B., Müllerné Trenovszki, M., Urbányi, B., . . . Müller, T. (2017). Habitat establishment, captive breeding and conservation translocation to save threatened populations of the Vulnerable European mudminnow Umbra krameri. Oryx, 51(4), 718-729. doi:10.1017/S0030605316000533.

Habitat establishment, captive breeding and conservation translocation to save threatened populations of the Vulnerable European mudminnow *Umbra krameri*

SÁNDOR TATÁR, BÁLINT BAJOMI, ANDRÁS SPECZIÁR, BALÁZS TÓTH, MAGDOLNA MÜLLERNÉ TRENOVSZKI, BÉLA URBÁNYI, BÉLA CSÁNYI, JÓZSEF SZEKERES and TAMÁS MÜLLER

SÁNDOR TATÁR*† Tavirózsa Association, Veresegyház, Hungary

BÁLINT BAJOMI† (Corresponding author) Faculty of Sciences, Centre for Environmental Sciences, Eötvös Loránd University, Pázmány Péter sétány 1/A, H-1117 Budapest, HungaryE-mail bb@greenfo.hu

ANDRÁS SPECZIÁR Balaton Limnological Institute, Hungarian Academy of Sciences Centre for Ecological Research, Tihany, Hungary

BALÁZS TÓTH Danube-Ipoly National Park Directorate, Budapest, Hungary

MAGDOLNA MÜLLERNÉ TRENOVSZKI, BÉLA URBÁNYI and TAMÁS MÜLLER† Department of Aquaculture, Szent István University, Gödöllő, Hungary

BÉLA CSÁNYI and JÓZSEF SZEKERES Danube Research Institute, Hungarian Academy of Sciences Centre for Ecological Research, Budapest, Hungary

*Also at: Department of Aquaculture, Szent István University, Gödöllő, Hungary

†These authors have contributed equally to the results presented in this paper.

Received 21 February 2016. Revision requested 4 March 2016. Accepted 5 May 2016.

Abstract In Europe 37% of freshwater fish are threatened. However, conservation activity is less widespread for fish compared to other vertebrate groups. The Vulnerable European mudminnow *Umbra krameri* is a marshland fish endemic to the Carpathian Basin. Its range and population have declined significantly since the 1990s. The main threats to the species are habitat loss and the invasive Chinese sleeper *Perccottus glenii*. During 2008–2012 a species conservation programme was established to rescue broodstocks from threatened populations, breed them under controlled conditions, translocate both rescued fish and their laboratory-reared offspring to surrogate habitats, and finally reintroduce offspring to their original habitats. Broodstocks from three threatened habitats were bred in the laboratory and produced offspring appropriate for stocking. Six artificial ponds were created in the pilot study area according to the environmental needs of the species, four of which proved to be suitable surrogate habitats in which translocated fish survived and reproduced successfully. Populations in the original habitats were supplemented with fish from laboratory breeding and from the natural recruitment of surrogate habitats, with special care of the corresponding broodstocks. Future challenges include improving our knowledge about the ecological processes in which the European mudminnow participates, identifying the most threatened populations, habitats suitable for restoration and potential areas for creation of surrogate habitats, and enhancing induced propagation methodology.

Keywords *Carassius carassius*, habitat loss, *Misgurnus fossilis*, reintroduction, surrogate habitats, threatened populations, *Umbra krameri*

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0000000000000000

Introduction

In their overall assessment of the European Red List of freshwater fish, Freyhof & Brooks (2011) concluded that nearly 80% of native species in Europe are endemic and 37% are threatened, which is high compared to other taxonomic groups (e.g. 23% of amphibians and 15% of mammals are threatened; Freyhof & Brooks, 2011). There is evidence that 13 fish species have already gone extinct in Europe, with five others facing impending extinction. The greatest threats to native European fish are pollution, habitat destruction and invasive alien species (Dudgeon et al., 2006). The effects of climate change (e.g. drought) have been shown to drive the deterioration of freshwater ecosystems, and fish living in shallow waters of wetlands are in particular danger (Heino et al., 2009; Pratchett et al., 2011; Jeppesen et al., 2012; Ellis et al., 2013). Nonetheless, fish are underrepresented in the conservation literature, with a clear bias in favour of birds and mammals (Seddon et al., 2005; Bajomi et al., 2010).

Fen habitats are particularly exposed to environmental regulation by people, and in Europe their area has decreased by 62% to 187,000 km^2 since the 1850s (Rosenthal et al., 1998; Joosten & Couwenberg, 2001; Brinson & Malvárez, 2002). In Hungary 97% of fens have been destroyed by regulation of watercourses, draining and ploughing (Tatár, 2010). Small, isolated remnant marshland populations are particularly sensitive to environmental alterations, which often lead to a decrease in genetic diversity and an increase in mortality, as in the case of the European mudminnow *Umbra krameri*. Freyhof & Brooks (2011) urged the establishment of Freshwater Key Biodiversity Areas and the development of species protection plans for European freshwater fish species. They also suggested establishing habitat monitoring and ex situ programmes, restrictions on settling of non-native fish species, and revision of the relevant legislation. One of the most important roles of a species protection plan is to identify and rectify factors that caused the initial extinction or decline (Fischer & Lindenmayer, 2000; IUCN/SSC, 2013; Cochran-Biederman et al., 2015).

Accordingly, we summarize the actions and results of our pilot conservation programme for the European mudminnow (Tatár et al., 2010).

The European mudminnow is small-bodied (7–8 cm), has a short lifespan (4 years), is the only native representative of the *Umbridae* family in Europe and is a relic and endemic fish species of the Danube catchment area (Gaudant, 2012). It occurs sporadically along the Danube River between Vienna and the Danube Delta. Some populations live in the lower stretches of the River Dniester but the species' main range is in the Carpathian Basin (Kottelat & Freyhof, 2007; Kuehne & Olden, 2014). The European mudminnow lives in marshes, fens, vegetated backwaters and channels with clean water (Pekárik et al., 2014). The main threats to the species are habitat loss as a result of dredging of channels, the destruction of river and stream floodplains, the loss of fens and marshes (Wanzenböck, 1995; Kuehne & Olden, 2014) and the spread of the invasive Chinese sleeper *Perccottus glenii*. This voracious competitor and predator of the European mudminnow is expanding its range in Eurasia and has colonized the catchment area of the Danube and Dniester rivers (Reshetnikov & Ficetola, 2011; Reshetnikov, 2013). It is estimated that populations of European mudminnow have declined by >30% (Freyhof, 2011). The species is categorized as Vulnerable on the IUCN Red List on the basis of its restricted and fragmented habitat (Freyhof, 2011) and it also features on the Red Lists of seven European countries (Müller et al., 2011).

Several successful attempts have been made to conserve populations of the species, primarily by means of habitat restoration and the reintroduction of rescued stocks (Wanzenböck, 1995; Keckeis & Sehr, 2014); for example, in the Slovenian Beloviči backwater populations of European mudminnow, as well as weatherfish *Misgurnus fossilis* and crucian carp *Carassius carassius*, increased significantly after restoration of their habitat (Povž, 1990). European mudminnow fry were also introduced successfully to waters in Austria (Benesch, 2004) and close to the River Morva in Slovakia, which is part of the species' native range (Valachovič & Kováč, 1998). However, translocations from Beloviči oxbow to a gravel pit (Povž 1995a) were unsuccessful. After the original population had gone extinct, the NGO Biotica Ecological Society restocked fry into small water bodies in the Lower Dniester area (Aps et al., 2004). A successful reintroduction was carried out in Fertő-Hanság National Park (north-west Hungary) by translocating wild individuals from other areas (Ambrus & Sallai, 2014; Ambrus pers. comm). Although some of these trials proved to be successful at a local scale, there is no detailed action plan for the conservation of declining populations of the European mudminnow across its diminishing and fragmented range.

Our aim was to develop and test a comprehensive methodology to promote the rescue and strengthening of populations of European mudminnow, for which habitat loss is one of the most important threats (Sallai, 2005; Kuehne & Olden, 2014). Considering the decreased population size, an important challenge is to preserve the species' remnant genetic diversity, distributed across small and isolated habitat patches (Takács et al., 2015). Accordingly, we implemented a pilot experiment that included:

(1) determining habitat characteristics required by the species, for consideration in the planning of surrogate habitats,

(2) creating new fen and marsh surrogate habitats to supplement lost native areas and harbour rescued populations (creating new self-sustaining populations in Szada Pilot Area),

(3) rescuing broodstocks from habitats known to be damaged or exposed to contamination,

(4) checking the quality of the created habitats before stocking them with European mudminnow,

(5) propagating and rearing European mudminnow under controlled laboratory conditions for reintroduction and for the conservation of genetic diversity,

and (6) monitoring the quality of the created habitats after stocking.

Behavioural degradation could be minimized by introducing rescued broodstock to the new surrogate habitats quickly rather than holding them in the laboratory (Hammer et al., 2012; Ellis et al., 2013). The key behavioural aspects for the survival of stocked fish are the ability to eat and avoid being eaten. Fish are often necessarily reared on artificial diets because of the cost, limited supply and potential disease risk of wild foods, but this potentially reduces their foraging efficiency in the wild (Hammer et al., 2012).

We hypothesized that carefully designed artificial habitats would be appropriate surrogate habitats for the European mudminnow to establish permanent self-sustaining stocks, and that either breeding in the laboratory or reproduction of broodstocks in the artificial habitats would yield enough offspring to reinforce threatened populations or replace extinct ones. To support the assessment of the ecological status of the created habitats, we included in the study two other threatened components of marshland fish assemblages, the weatherfish *Misgurnus fossilis* and the crucian carp *Carassius carassius*.

Study area

For habitat construction experiments we selected nine natural lowland sites within Hungary's Carpathian Basin (Fig. 1;Table 1) that had existing or previously known populations of European mudminnow (Sallai, 2005). The chosen habitats represented the environmental range occupied by the species, and included fens, ponds, slow-flowing and vegetated streams, and canals. Two more sites were added subsequently because of threats to the species: Gőgő-Szenke Stream, which was threatened by anthropogenic pollution, and a fen in the construction area of the South M0 highway bridge (Ráckeve Danube Branch Natura 2000 Site, Czuczor Island), which was about to be filled up as part of the expansion of the highway.

The 20 ha Szada Pilot Area (Fig. 1) was chosen for the creation of new and revitalized fen and marsh habitats (Illés Ponds I–VI) based on the following criteria. Firstly, it is a drained wetland area with a few small and isolated native populations of European mudminnow (in Pócos Ponds A and B, sampling sites 8 and 9; Fig. 1). Secondly, the groundwater level does not drop below 1.5 m even during droughts, thus facilitating the creation and maintenance of fen and marsh habitats. Thirdly, this area is adjacent to the EU's Natura 2000 network of protected areas (Natura 2000, 2012), and thus there should be no further risk of significant human impact. Finally, it is close to the laboratory of Szent István University (Gödöllő) where breeding took place.

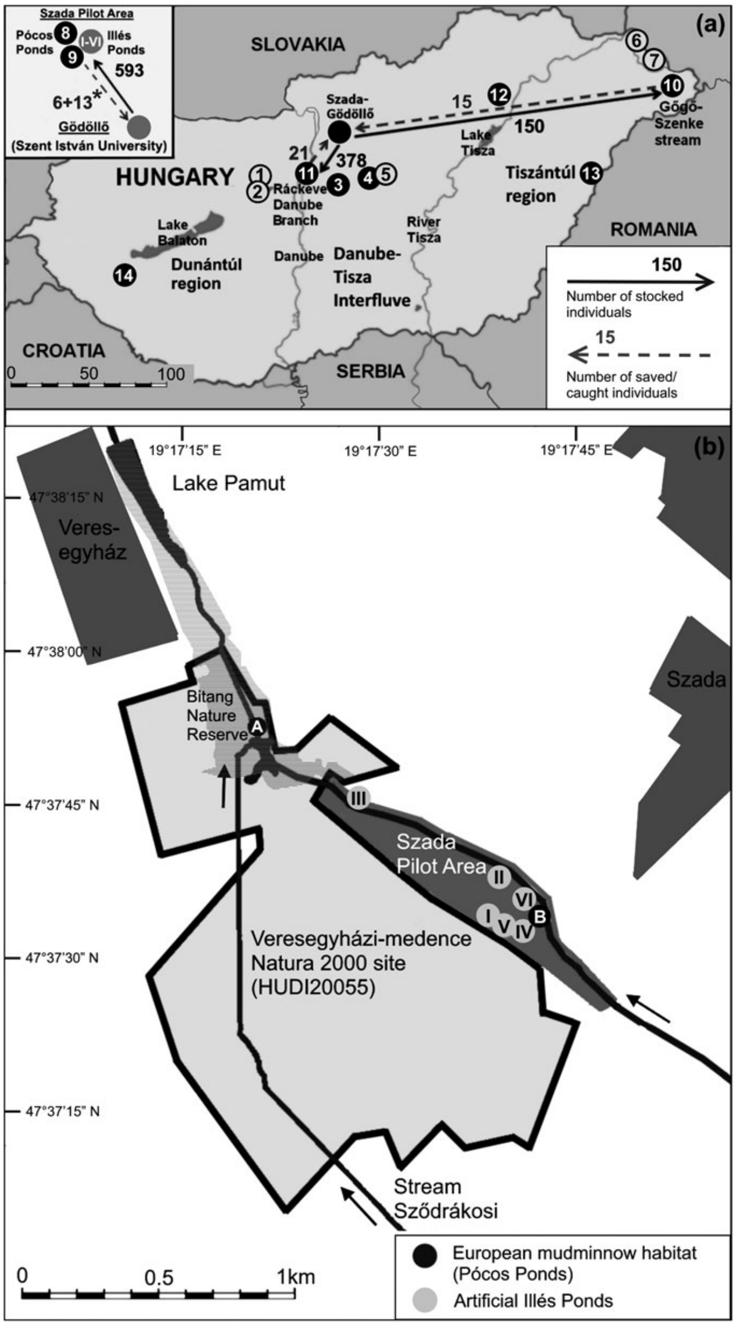

FIG. 1(a) Sites of surveyed European mudminnow *Umbra krameri* populations and habitats (Table 1 and Table S2), number of caught mudminnows and stocked captive bred individuals in Hungary. White circles represent extinct populations. Inset shows number of caught fish for propagation and stocked offspring in Illés Ponds I, III, IV, VI of Szada Pilot Area (Table 3).

(b) Location of natural and artificial ponds (Pócos and Illés Ponds, respectively) in Szada Pilot Area.

* Parent fish were captured in Pócos Pond B (site 9; 6 ind.) and Illés Pond IV (13 ind.). Latter habitat is connected to Pócos Pond B.

Methods

Assessment of habitats and new surrogate habitats (Illés Ponds)

The ecological requirements of the European mudminnow were determined based on a comprehensive literature review and field research in native habitats. In total, 11 natural habitats of the species in the three main regions of Hungary were selected at random for surveys, from a habitat list of National Parks (Fig. 1).

Water quality analysis and botanical investigations were conducted between February and September, and hydrobiological surveys were carried out in May and June during 2008–2015. Dissolved oxygen, pH and conductivity were recorded using portable water quality meters (Voltcraft DO-100, PH-100 ATC and LWT-01, Conrad Electronic, Hirschau, Germany) and other chemical properties of the water (phosphate, ammonium, nitrite and nitrate ion concentrations) were measured using a VISOCOLOR® ECO test kit (Macherey-Nagel, Dűren, Germany). Abundance and number of taxa of macroinvertebrates (i.e. food base of fish) were assessed using a kick-and-sweep sampling method (mesh size 950 µm, frame size 25×25 cm). Macroinvertebrate samples were preserved in 4% formaldehyde or 96% ethanol prior to identification in a laboratory. Botanical investigations included assessment of the macrophyte coverage and identification of the dominant taxa at each site. Algal or bacterial blooms observed were recorded.

Fish assemblages were sampled during 2008–2012 using electric fishing (IG200, Hans Grassl, Schönau am Königssee, Germany) along randomly selected 150 m long transects. Samples were taken by wading upstream in streams and canals, and covering the whole volume of small, still waters (i.e. ponds and fens; <500 m^3). Captured fish were identified immediately, counted and released. The same procedure and methodology used for natural habitats was also used to monitor the pre- and post-stocking water quality of Illés Ponds.

Creation and monitoring of new surrogate habitats

Based on a literature review and field surveys of the natural habitats of the European mudminnow (Supplementary Tables S1–S5) we designed Illés Ponds in the Szada Pilot Area (Fig. 1). During 2008–2010 we established six groundwater-fed ponds (i.e. Illés Ponds I–VI; each with 50–60 m^3 volume, 30–40 m^2 surface area, 1–1.5 and 2.5 m mean and maximum depths, respectively) by dredging degraded terrestrial habitats dominated by the invasive plants *Solidago* spp. Rather than creating one large lake we designed several smaller ponds with a high shoreline-to-surface ratio, which is important for the development of diverse and abundant macrophyte and macroinvertebrate assemblages (Gee et al., 1997; Cremona et al., 2008). To increase environmental diversity we created irregular shorelines and bottoms. The ponds were created such that 50−70% of their surface area was in the shade of the surrounding trees and shrubs to prevent excessive warming and algal blooms, which are not tolerated by marshland fish. Shading was increased by introducing macrophytes to the ponds. Aquatic vegetation also decreases the nitrate, nitrite and ammonium content of water, which also inhibits the growth of algae. We planted the ponds with indigenous aquatic macrophytes *Ceratophyllum demersum* and *Lemna minor* from a nearby water body; the common reed *Phragmites australis* was present in the Szada Pilot Area and colonized the littoral of Illés Ponds spontaneously. As we wished to preserve the genetic identity of each of the rescued broodstocks, Illés Ponds were constructed in such a way as to ensure the isolation from each other and from the surrounding aquatic habitats (except Illés Pond IV, which was created as a refuge extension of the over-vegetated and shallow natural Pócos Pond B). Isolation of experimental ponds from surrounding watercourses also prevents immigration of invasive fish.

After their construction Illés Ponds were monitored regularly for water chemistry, macrophytes and macroinvertebrates (Supplementary Tables S1–S5). Following 8–23-month colonization periods, ponds that were considered to be appropriate were stocked with European mudminnow. After stocking, monitoring of ponds was supplemented with seasonal sampling of fish, applying the same method outlined above for small, still water bodies.

Saving threatened stocks, captive breeding and releases

During our field trips and by maintaining contact with other conservationists countrywide we actively searched for situations where populations of European mudminnow were threatened by human activities. In such cases, fish were captured using electric fishing, placed in a plastic barrel filled with oxygenated water and transported to a laboratory at the Szent István University.

In the laboratory broodstocks were held in separate aquaria to preserve their genetic identity for a 2-week acclimatization period prior to attempted breeding using two procedures. The first group was placed in breeding tanks with 15°C water temperature and a photoperiod similar to that of the spawning season, in April; the bottom of the tanks was covered by a green plastic net to prevent cannibalism of eggs and larvae. (Green netting was used for camouflage amidst the aquatic vegetation.) The second group received carp pituitary extract treatment to induce propagation. Larvae were then reared in aquaria and in an artificial 10 m^3 pond lined with foil. For more details of propagation and rearing see Müller et al. (2011) and Demény et al. (2014).

Captured fish, and their laboratory-reared offspring, originating from different populations were stocked in separate Illés Ponds. Offspring from captive breeding and from natural spawning in Illés Ponds were used to supplement populations in native habitats where the parents originated. Laboratory-reared weatherfish and crucian carp were also stocked in Illés Ponds.

Results

Environmental characteristics of European mudminnow habitats According to published data and the results of our surveys the physical and chemical water quality of natural habitats of the European mudminnow varies widely (Supplementary Table S1). The species' habitats usually have a low dissolved oxygen concentration; for example, we measured oxygen levels of 0.87 and 0.31 mgl^{-1} in Pócos Pond A and at Ócsa Landscape Protection Area, respectively. Our field surveys revealed that macrophyte coverage in these habitats can vary widely (0–100%), with a mean value of 61% (Table 1). The European mudminnow generally occurs in shallow (0.5–1.5 m), shaded and often low-volume waterbodies. Data on the abundance of macroinvertebrates in samples from various native habitats are summarized in Supplementary Table S2.

Fish assemblages of European mudminnow habitats Of the nine investigated sites where the presence of the European mudminnow had been confirmed previously, we found the species only at four (Table 1). We also found the species at the two additional sites threatened by anthropogenic impacts (i.e. the fen at the South M0 Bridge and Gőgő-Szenke Stream). In general, fish assemblages of still-water habitats comprised fewer species (1–4) than those of streams and canals (2–11; Table 1). There was a negative relationship between the occurrence of the European mudminnow and the presence of invasive species. We found the European mudminnow in abundance only at five sites that lacked invasive species or had a low abundance of Prussian carp *Carassius gibelio* (i.e. Gőgő-Szenke Stream). In contrast, five former habitats of the European mudminnow where the species is no longer present are now populated by invasive fish, including the black bullhead *Ameiurus melas*, Prussian carp, pumpkinseed *Lepomis gibbosus*, Chinese sleeper *Perccottus glenii* and stone moroko *Pseudorasbora parva*. We found only one exception; the Felső-Tápió stream harboured a small number of European mudminnow as well as an abundance of invasive species. The weatherfish was the most common native species captured over all sites.

Creation and pre-stocking monitoring of surrogate habitats Pre-stocking water quality, macrophyte and macroinvertebrate (i.e. food base) monitoring (Supplementary Tables S1–S3) revealed that Illés Ponds I, III, IV and VI met the species' conservation criteria following a short primary succession period, and thus they were assigned for stocking with European mudminnow during 2010–2011. In Illés Pond II there were regular blooms of cyanobacteria, and proliferation of sulphur bacteria was also a common phenomenon. In 2012 we observed high nitrite concentrations (Supplementary Table S1) and blooms of *Cladophora* sp. in Illés Pond V. Consequently Illés Ponds II and V were excluded from the conservation programme.

Captive breeding and saving threatened stocks In 2010 a total of 42 adult European mudminnow were rescued and transported to the laboratory from three threatened sites: the Pócos Pond B (site 9), which nearly dried up in that year; the heavily polluted Gőgő-Szenke Stream (site 10); and the fen in the construction area of the South M0 Bridge (site 11; ×Table 2). Induced propagation with carp pituitary treatment was attempted three times but no larvae were hatched. In contrast, fish that did not receive any treatment formed bonded pairs and laid eggs on the bottom of the breeding tank. From this 864 juveniles were introduced to the Szada Pilot Area and the parents' original habitat (×Table 3). Laboratory-reared juveniles reached a mean standard body length of 4.7 cm in aquaria by early August; they reached a mean body length of 5.5 cm and sexual maturity (females visibly carried developing eggs) in an artificial pond by mid September.

Stocking surrogate habitats with marshland fish Environmental assessment of Illés Pond I 14 months after its creation indicated favourable conditions for marshland fish (Tables S1 & S3). However, as we had not yet rescued any European mudminnow at that time and therefore we stocked 50 one-summer-old juveniles of weatherfish and crucian carp in the pond in autumn 2009. Test fishing in 2010 revealed that these fish had survived in the pond (×Table 4). We subsequently stocked Illés Ponds I, III, IV and VI with European mudminnow. After propagation in the laboratory we released a total of 20 saved adult European mudminnow into Illés Ponds I, III and IV in spring 2010 and summer 2011 (Table 2). Between spring 2010 and summer 2012 we stocked Illés Ponds I, III, IV and VI with 593 laboratory-reared juveniles aged 43–188 days (Table 3). During 2011–2012 we stocked Illés Ponds III and VI with 220 weatherfish and 108 crucian carp juveniles, respectively, which had been bred under laboratory conditions.

Post-stocking monitoring of surrogate habitats Results of water quality and macroinvertebrate assessments following the stocking of Illés Ponds I, III, IV and VI with European mudminnow are in Supplementary Tables S4 and S5. Monitoring revealed that water quality, number of macroinvertebrate taxa, and macroinvertebrate abundance of these ponds are mainly within the range of natural habitats. Test fishing showed that introduced fish survived in their new habitats. Observed mean recapture rates were 17% for European mudminnow, 48% for crucian carp and 13% for weatherfish (Table 4). Natural reproduction of European mudminnow was evident in the year following the first introductions (Table 4). Recruitment was most abundant in the densely vegetated Illés Pond III. Although we released more adults into Illés Pond I (n=13) than into Illés Pond III (n=5), reproduction success was weaker in the former, probably because of the low macrophyte coverage.

Reinforcement of natural European mudminnow populations Given the success of our experiments we were able to reinforce and reintroduce populations of European mudminnow in the threatened habitats where parent fish had originated. Following an administrative measure to eliminate pollution we reintroduced 100 39-day-old and 50 208-day-old laboratory-reared individuals into Gőgő-Szenke Stream in May and October 2010, respectively (Table 3). Laboratory-reared 429-day-old offspring (n=121) of parents that originated from the fen at the South M0 Bridge were introduced to a similar, nearby habitat, Ráckeve Danube Branch at Szigetcsép (Table 3). Following successful spawning of European mudminnow, offspring were partially harvested from Illés Pond III and 257 individuals of c. 131 and 180 days old were reintroduced to the preserved remnant of their native habitat, the fen at the South M0 Bridge, in August and September 2011, respectively. The threatened population of Pócos Pond B was reinforced indirectly by ensuring its connectivity with Illés Pond IV.

Discussion

Environmental characteristics of European mudminnow habitats

We recorded extremely low dissolved oxygen concentrations in several habitats. This circumstance is tolerated by the European mudminnow, which uses its swim-bladder as an auxiliary breathing organ (Geyer & Mann, 1939). The ability to survive periods of low oxygen concentrations confers a competitive advantage over other fish species in marsh and fen environments.Pekárik et al. (2014) found that both the probability of the presence of European mudminnow and its abundance tend to increase with macrophyte coverage. The mean value of macrophyte coverage was 52 and 9% (free-floating and submerged plants, respectively) in the surveyed habitats, which differs from the assessment of Pekárik et al. (2014), who concluded that the optimal macrophyte coverage is c. 45 and 39%.

Current status of native European mudminnow populations

More than half (five of the nine investigated) of the previously known populations were not found in our surveys, indicating the species may be at considerable risk of extinction. Other studies have reached similar conclusions (Sallai, 2005; Kuehne & Olden, 2014) and identified two main sources of threats. Most of the species' habitat was lost during the extensive regulation of rivers and wetlands in the 19th and 20th centuries (Takács et al., 2015). However, habitat loss is ongoing, as illustrated by the case of the fen at the South M0 Bridge, where construction work related to transport infrastructure and expansion of recreational areas are resulting in the loss of wetland habitats.

The second evident threat is the spread of invasive fish species. Invasive species were abundant in all former habitats of the European mudminnow where we failed to capture any individuals. Conversely, where the European mudminnow was present invasive species were generally absent or occurred only in small numbers. The most voracious invader in these habitats is the Chinese sleeper, which is known to have negative impacts on the European mudminnow (Sallai, 2005; Reshetnikov, 2013). The European mudminnow may coexist with most invasive fish species (Keresztessy, 1995; Povž, 1995a) but in the long term it does not survive the settlement of the Chinese sleeper (Sallai, 2005; Reshetnikov, 2013). Although there are signs of both competition from and predation by other invasive species (Ferincz et al., 2014), the extent of these relationships has not yet been quantified. It is likely that, at least in some cases, invasive species are not the primary cause of the European mudminnow's population decline. The proliferation of invasive species in wetland habitats could be an indicator of environmental degradation (e.g. regulated water level, dredging of macrophytes, increased nutrient load), and thus their effect on the European mudminnow could be considered to be indirect. Habitats of the European mudminnow generally contain species-poor fish assemblages; among the most common associate species are the weatherfish and the crucian carp (Sallai, 2005; Pekárik et al., 2014), which are adapted to survive periods of extremely low oxygen concentrations and low water levels (Geyer, 1940; Povž, 1995b). Habitat alteration may facilitate the establishment of other species, including invasive fishes and, therefore, it is important to conserve and restore wetland habitats in their original form.

Both refuge fen habitats in the Szada Pilot Area (i.e. Pócos Ponds A and B) contained European mudminnow populations and were free of invasive species, which supports the relevance of this area for a species conservation experiment.

Establishment and pre-stocking monitoring of surrogate habitats

Illés Ponds, created based on our experiences studying native habitats of the European mudminnow, revealed variable environmental characteristics. Although these ponds are located close together in the same degraded wetland landscape, they vary in their water quality, macrophyte flora and macroinvertebrate fauna. Four of the ponds proved to be suitable for marshland fish conservation, being occupied by rich macroinvertebrate, amphibian and reptilian assemblages, including rare and protected species (e.g. the raft spider *Dolomedes fimbriatus*, the smooth newt *Lissotriton vulgaris*, the common spadefoot *Pelobates fuscus*, the grass snake *Natrix natrix* and the European pond turtle *Emys orbicularis*).

The creation of several small habitat patches (i.e. ponds) instead of a more extended and connected fen system has several advantages, especially in monitoring and controlling ongoing biological and environmental processes, preventing the spread of disease, and keeping invasive species out of the system. It also facilitates the preservation and maintenance of broodstocks from different populations with unique genetic pools (Marić et al., 2015; Takács et al., 2015).

Pre-stocking monitoring of created habitats is advisable, as habitat quality and prey availability influence the outcome of introductions of freshwater fish (Griffith et al., 1989;

Ellis et al., 2013; Cochran-Biederman et al., 2015). The unfavourable conditions in Illés Ponds II and V may be attributable to the soil structure and the slow flux of groundwater. Furthermore, shading from riparian vegetation could prohibit the establishment of macrophytes, thus facilitating planktonic eutrophication.

Saving fish from threatened habitats

Our study targeted three threatened populations of European mudminnow: the fen at the South M0 Bridge construction area and the Gőgő-Szenke Stream, both of which had suffered direct anthropogenic impacts, and Pócos Pond B, where habitat loss had occurred as a result of drought. We believe that at least the first two might have gone extinct without our action. Rapid rescue and relocation of broodstocks from these sites to the laboratory and to surrogate habitats created in the Szada Pilot Area ensured the survival of these gene pools.

Rescuing and maintaining broodstocks from threatened populations is a commonly used conservation tool (Hammer et al., 2012; Ellis et al., 2013). However, captive maintenance may raise problems of reduced genetic diversity given the small number of parent fish (Philippart, 1995), and behavioural alterations under laboratory conditions (Philippart, 1995; Lynch & O'Hely, 2001). Nevertheless, many wild populations of European mudminnow could be small, especially during droughts. It should be emphasized that many of these isolated populations may represent unique gene pools and therefore important management units (Takács et al., 2015).

Captive breeding

Reintroductions of threatened species depend on successful captive-breeding programmes (Witzenberger & Hochkirc, 2011). The majority of freshwater fish species can be propagated using hormone treatments, even out of their reproductive season (Muscalu-Nagy et al., 2011; Zakęśet al., 2013). However, all our attempts to induce controlled propagation of European mudminnow failed. Despite there being no reports of successful induced propagation in this species, this outcome is still surprising, particularly as fish spawned spontaneously in the aquaria under temperature and light conditions similar to those in the spawning season. Spontaneous spawning of European mudminnow has also been observed by other researchers (Geyer, 1940; Povž, 1990; Bohlen, 1995; Kováč, 1997; Müller et al., 2011; Demény et al., 2014; Kucska et al., 2016). Consequently, laboratory-bred offspring may be used for species conservation purposes but their availability could be limited both in quantity and timing. We obtained a sufficient quantity of larvae from laboratory spawning to stock the Illés Ponds, and the larvae were easily reared to sizes suitable for stocking.

Survival of fish in surrogate habitats

The most critical measure of any surrogate habitat is whether the introduced species can survive and reproduce there (i.e. establish a self-sustaining population; Fischer & Lindenmayer, 2000; Cochran-Biederman et al., 2015). Although we only monitored a limited post-stocking period, the results suggest that stocked European mudminnow, as well as weatherfish and crucian carp, survived in the four Illés Ponds that were initially determined to be suitable environments for fish conservation. Reproductive success was also evident in Illés Ponds I and III. In Illés Pond IV we also observed some offspring; however, as this pond is connected to native Pócos Pond B, the exact place of spawning could not be identified. In the case of Illés Pond VI, post-stocking monitoring did not cover a sufficiently long period to evaluate reproductive success. However, it should be emphasized that in densely vegetated habitats such as Illés Ponds, fish sampling is ineffective and results are biased, as electronarcotized individuals, especially small-bodied juveniles, remain hidden among the dense macrovegetation. Furthermore, considering the small size of stocked individuals recapture rates probably significantly underestimated actual survival rates.

Illés Ponds were designed for permanent use, with introduced broodstocks to be maintained without further disturbance except regular monitoring. Long-term monitoring of these populations will ultimately reveal whether our ex situ conservation experiment has been successful; however, benefits of Illés Ponds are already apparent in that we were able to harvest offspring hatched there and reintroduce them to native habitats.

Reinforcement of natural populations

The ideal outcome for a conservation rescue is that threatened and rescued populations recover in their original restored habitat or in an equivalent natural or artificial surrogate habitat within the range of the conservation unit (Fischer & Lindenmayer, 2000; Olden et al., 2011; Ellis et al., 2013). We deemed our efforts to save and conserve populations of European mudminnow as successful when we reintroduced all three threatened populations to their original habitats while preserving their genetic identity.

Conclusion

Populations of European mudminnow have declined or disappeared over most of their native range and are threatened by habitat loss and invasive species. Our pilot programme is the first comprehensive species conservation programme that includes rescuing individuals from threatened populations, captive breeding and rearing, creation of surrogate habitats, introduction of saved and captive-bred stocks and reinforcement of threatened parent populations of European marshland fish. Our experience in the conservation of the European mudminnow has been positive and our results attest to the wide-scale relevance of such complex approaches in preserving other wetland species and biodiversity. However, to facilitate larger-scale conservation actions further research is needed to resolve the problem of reliable induced propagation in this species. In addition range-wide investigations are needed to improve our knowledge of the ecological processes in which the European mudminnow participates and to identify the most threatened populations, habitats for restoration and potential areas for creation of new surrogate habitats. Conservation actions should be synchronized and a range-wide conservation plan should be developed, taking into consideration the genetic guide on relevant conservation and management units, based on information about which populations may be used for recruiting (i.e. serving a pool of parent fish in artificial breeding programmes) and stocking into reconstructed habitats in different geographical areas, and which populations have the genetic integrity to be preserved without mixing them with other populations (Takács et al., 2015).

Acknowledgements

We are grateful to Bence Balován (Tavirózsa Association), Zsolt Boczonádi (Szent István University), György Hajdú (Umbra Association), Katalin Keresztessy (Szent István University), Sándor Krenedits (Tavirózsa Association), David McFetridge (London School of Business and Finance), Zoltán Sallai (Hortobágy National Park Directorate), Dávid Szőts (Nagy Gáspár City Library), Dr Péter Takács (Balaton Limnological Institute), Dr András Takács-Sánta (Eötvös Loránd University), László Vécsey (Municipality of Szada), Prof. Dr Gábor Vida (Eötvös Loránd University) and Gábor Várbíró (Hungarian Academy of Sciences). We thank Doug Armstrong (Massey University), Robin Pakeman (James Hutton Institute), Daniel L. Jeffries (University of Lausanne) and Nathan Spillman (Society for Conservation Biology) for their useful comments.

Support from Magyar Telekom Nyrt. helped to start the European Mudminnow Conservation Pilot Programme. Our research was funded by the Hungarian Ministry of Environment and Water and the Hungarian Ministry of Rural Development (Green Source Programme 2008–2009, 2011–2012), the National Innovation Office, and Research Centre of Excellence-9878/2015/FEKUT.

Author contributions

ST conducted the water quality and botanical investigations and planned the habitat reconstruction and assessment. BB assisted with summarizing the programme results and writing the manuscript. AS analysed the water quality and botanical data. BT carried out fish fauna studies and analysed the results. MMT and TM carried out captive breeding and rearing of European mudminnow, European weatherfish and crucian carp. BU analysed the data on the macroinvertebrate and fish fauna. BC and JS carried out surveys of macroinvertebrate fauna.

References

AMBRUS, A. & SALLAI, Z. (2014) Distribution and conservation of the European mudminnow (*Umbra krameri* Walbaum, 1792) at the range of the Fertő-Hanság National Park (Northwest Hungary). *Pisces Hungarici*, 8, 97–100. [In Hungarian with English abstract]

APS, R., SHARP, R. & KUTONOVA, T. (2004) *Freshwater Fisheries in Central & Eastern Europe: The Challenge of Sustainability*. Overview Report. IUCN, Warsaw, Poland.

BAJOMI, B., PULLIN, A.S., STEWART, G.B. & TAKÁCS-SÁNTA, A. (2010) Bias and dispersal in the animal reintroduction literature. *Oryx*, 44, 358–365.

BENESCH, A.R. (2004) Wiedereinbürgerung Hundsfisch (*Umbra krameri* W.) im österreichischen Teil des Hanság/Burgenland. *Österreichs Fischerei*, 57, 161–165.

BOHLEN, J. (1995) Laboratory studies on the reproduction of the European mudminnow, *Umbra krameri* Walbaum 1792. *Annalen des Naturhistorischen Museums in Wien*, 97, 502–507.

BRINSON, M.M. & MALVÁREZ, A.I. (2002) Temperate freshwater wetlands: types, status and threats. *Environmental Conservation*, 29, 115–133.

COCHRAN-BIEDERMAN, J.L., WYMAN, K.E., FRENCH, W.E. & LOPPNOW, G.L. (2015) Identifying correlates of success and failure of native freshwater fish reintroductions. *Conservation Biology*, 29, 175–186.

CREMONA, F., PLANAS, D. & LUCOTTE, M. (2008) Biomass and composition of macroinvertebrate communities associated with different types of macrophyte architectures and habitats in a large fluvial lake. *Fundamental and Applied Limnology*, 171, 119–130.

DEMÉNY, F., MÜLLERNÉ TRENOVSZKI, M., TATÁR, S., SIPOS, S., URBÁNYI, B. & MÜLLER, T. (2014) Effect of feeding frequency on the growth of the European mudminnow larvae (*Umbra krameri* Walbaum, 1792) reared in controlled conditions. *Bulgarian Journal of Agricultural Science*, 20, 670–674.

DUDGEON, D., ARTHINGTON, A.H., GESSNER, M.O., KAWABATA, Z.-I., KNOWLER, D.J., LÉVÊQUE, C.et al. (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, 81, 163–182.

ELLIS, I.M., STOESSEL, D., HAMMER, M.P., WEDDERBURN, S.D., SUITOR, L. & HALL, A. (2013) Conservation of an inauspicious endangered freshwater fish, Murray hardyhead (*Craterocephalus fluviatilis*), during drought and competing water demands in the Murray-Darling Basin, Australia. *Marine & Freshwater Research*, 64, 792–806.

FERINCZ, Á., STASZNY, Á., WEIPERT, A., SÜTŐ, A., SOCZÓ, G., ÁCS, A. et al. (2014) Adatok a dél-balatoni berekterületek halfaunájához. *Natura Somogyiensis*, 24, 279–286. [In Hungarian]

FISCHER, J. & LINDENMAYER, D.B. (2000) An assessment of the published results of animal relocations. *Biological Conservation*, 96, 1–11.

FREYHOF, J. (2011) *Umbra krameri*. The IUCN Red List of Threatened Species 2011: e.T22730A9380477. Http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T22730A9380477.en [accessed 21 January 2016].

FREYHOF, J. & BROOKS, E. (2011) *European Red List of Freshwater Fishes*. Publications Office of the European Union, Luxembourg.

GAUDANT, J. (2012) An attempt at the palaeontological history of the European mudminnows (Pisces, Teleostei, Umbridae). *Neues Jahrbuch für Geologie und Paläontologie*, 263, 93–109.

GEE, J.H.R., SMITH, B.D., LEE, K.M. & GRIFFITHS, S.W. (1997) The ecological basis of freshwater pond management for biodiversity. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 7, 91–104.

GEYER, F. (1940) Der ungarische Hundsfisch *(Umbra lacustris* Grossinger). *Zeitschrift für Morphologie und Ökologie der Tiere*, 36, 745–811.

GEYER, F. & MANN, H. (1939) Beiträge zur Atmung der Fische I. Die Atmung des Ungarischen Hundsfisches (*Umbra lacustris* GROSSINGER). *Zoologischer Anzeiger*, 127, 234–245.

GRIFFITH, B., SCOTT, J.M., CARPENTER, J.W. & REED, C. (1989) Translocation as a species conservation tool: status and strategy. *Science*, 245, 477–480.

HAMMER, M., BARNES, T., PILLER, L. & SORTINO, D. (2012) *Reintroduction Plan for the Purple-spotted Gudgeon in the Southern Murray–Darling Basin*. MDBA Publication No. 45/12. Murray–Darling Basin Authority, Canberra, Australia.

HEINO, J., VIRKKALA, R. & TOIVONEN, H. (2009) Climate change and freshwater biodiversity: detected patterns, future trends and adaptations in northern regions. *Biological Reviews*, 84, 39–54.

IUCN/SSC (2013) *Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0*. IUCN Species Survival Commission, Gland, Switzerland.

JEPPESEN, E., MEHNER, T., WINFIELD, I.J., KANGUR, K., SARVALA, J., GERDEAUX, D. et al. (2012) Impacts of climate warming on the long-term dynamics of key fish species in 24 European lakes. *Hydrobiologia*, 694, 1–39.

JOOSTEN, H. & COUWENBERG, J. (2001) Bilanzen zum Moorverlust. Das Beispiel Europa. In *Landschaftsökologische Moorkunde,* 2nd edition (eds M. Succow & H. Joosten), pp. 406–409. Schweizerbart, Stuttgart, Germany.

KECKEIS, H. & SEHR, M. (2014) Vorkommen und Verteilung des Hundsfisches (*Umbra krameri*, Walbaum, 1792) im Fadenbach im Bereich Mannsdorf an der Donau bis Witzelsdorf. *Wissenschaftliche Reihe Nationalpark Donau-Auen*, 36, 1–67.

KERESZTESSY, K. (1995) Recent fish faunistical investigations in Hungary with special reference to *Umbra krameri* Walbaum, 1792 (Pisces: Umbridae). *Annalen des Naturhistorischen Museums in Wien*, 97, 458–465.

KOTTELAT, M. & FREYHOF, J. (2007) *Handbook of European Freshwater Fishes*. Publications Kottelat, Cornol, Switzerland.

KOVÁČ, V. (1997) Experience with captive breeding of the European mudminnow, *Umbra krameri* Walbaum, and why it may be in danger of extinction. *Aquarium Sciences and Conservation*, 1, 45–51.

KUCSKA, B., KABAI, P., HAJDÚ, J., VÁRKONYI, L., VARGA, D., MÜLLERNÉ-TRENOVSZKI, M. et al. (2016). Ex situ protection of the European mudminnow (*Umbra krameri* Walbaum, 1792): spawning substrate preference for larvae rearing under controlled condition. *Archives of Biological Sciences*, 68, 61–66.

KUEHNE, L.M. & OLDEN, J.D. (2014) Ecology and conservation of mudminnow species worldwide. *Fisheries*, 39, 341–351.

LYNCH, M. & O'HELY, M. (2001) Captive breeding and the genetic fitness of natural populations. *Conservation Genetics*, 2, 363–378.

MÁJSKY, J. & HAJDÚ, J. (2004) *Program Záchrany Blatniaka Tmavého (*Umbra krameri *Walbaum, 1792)*. Správa CHKO Dunajské luhy. [In Slovak]

MARIĆ, S., SNOJ, A., SEKULIĆ, N., KRPO-ĆETKOVIĆ, J., ŠANDA, R. & JOJIĆ, V. (2015) Genetic and morphological variability of the European mudminnow *Umbra krameri* (Teleostei, Umbridae) in Serbia and in Bosnia and Herzegovina, a basis for future conservation activities. *Journal of Fish Biology*, 86, 1534–1548.

MÜLLER, T., BALOVÁN, B., TATÁR, S., MÜLLERNÉ-TRENOVSZKI, M., URBÁNYI, B. & DEMÉNY, F. (2011) A lápi póc (*Umbra krameri*) szaporítása és nevelése a természetesvízi állományok fenntartása és megerősítése érdekében. *Pisces Hungarici*, 5, 15–20. [In Hungarian, with English abstract]

MUSCALU-NAGY, C., APPELBAUM, S. & GOSPIČ, D. (2011) A new method for out-of-season propagation for northern pike (*Esox Lucius*, L.). *Animal Science and Biotechnologies*, 44, 31–34.

NATURA 2000 (2012) HUDI20055: Veresegyházi-medence. Http://natura2000.eea.europa.eu/Natura2000/SDF.aspx?site=HUDI20055 [accessed 21 January 2016].

OLDEN, J.D., KENNARD, M.J., LAWLER, J.J. & POFF, N.L. (2011) Challenges and opportunities in implementing managed relocation for conservation of freshwater species. *Conservation Biology*, 25, 40–47.

PEKÁRIK, L., HAJDÚ, J. & KOŠČO, J. (2014) Identifying the key habitat characteristics of threatened European mudminnow (*Umbra krameri* Walbaum 1792). *Fundamental and Applied Limnology*, 184, 151–159.

PHILIPPART, J.C. (1995) Is captive breeding an effective solution for the preservation of endemic species? *Biological Conservation*, 72, 281–295.

POVŽ, M. (1990) Conservation of the mudminnow, *Umbra krameri* Walbaum, in Slovenia. *Journal of Fish Biology*, 37, 243–245.

POVŽ, M. (1995a) Discovery, distribution, and conservation of mudminnow *Umbra krameri* Walbaum, 1792, in Slovenia (Pisces: Umbridae). *Annalen des Naturhistorischen Museums in Wien*, 97, 478–485.

POVŽ, M. (1995b) Threatened fishes of the world: *Umbra krameri* Walbaum, 1792 (Umbridae). *Environmental Biology of Fishes*, 43, 232.

PRATCHETT, M.S., BAY, L.K., GEHRKE, P.C., KOEHN, J.D., OSBORNE, K., PRESSEY, R.L. et al. (2011) Contribution of climate change to degradation and loss of critical fish habitats in Australian marine and freshwater ecosystems. *Marine and Freshwater Research*, 62, 1062–1081.

RESHETNIKOV, A.N. (2013) Spatio-temporal dynamics of the expansion of rotan *Perccottus glenii* from West-Ukrainian centre of distribution and consequences for European freshwater ecosystems. *Aquatic Invasions*, 8, 193–206.

RESHETNIKOV, A.N. & FICETOLA, G.F. (2011) Potential range of the invasive fish rotan (*Perccottus glenii*) in the Holarctic. *Biological Invasions*, 13, 2967–2980.

ROSENTHAL, G., HILDEBRANDT, J., ZÖCKLER, Ch., HENGSTENBERG, M., MOSSAKOWSKI, D., LAKOMY, W. & BURFEINDT, I. (1998) Feuchtgrünland in Norddeutschland—Ökologie, Zustand, Schutzkonzepte. Erarbeitung von Biotopschutzkonzepten der Bundesrepublik Deutschland für ausgewählte Biotoptypen: Feuchtgrünland. In *Angewandte Landschaftsökologie* (eds R. Schneider-Sliwa, D. Schaub & G. Gerold). Bundesamt für Naturschutz, Schriftenvertrieb im Landwirtschaftsverlag, Münster, Germany.

SALLAI, Z. (2005) A lápi póc (*Umbra krameri*) magyarországi elterjedése, élőhelyi körülményeinek és növekedési ütemének vizsgálata a kiskunsági Kolon-tóban. In *A Puszta* (ed. T. Barna), pp. 113–172. Nimfea Természetvédelmi Egyesület, Túrkeve, Hungary. [In Hungarian]

SALLAI, Z. & MÜLLER, T. (2014) A lápi póc. In *Veszélyeztetett lápi halak megóvása (lápi póc, réticsík, széles kárász)* (ed. T. Müller), pp. 11–84. Vármédia Print Kft, Gödöllő, Hungary. [In Hungarian]

SEDDON, P.J., SOORAE, P.S. & LAUNAY, F. (2005) Taxonomic bias in reintroduction projects. *Animal Conservation*, 8, 51–58.

SEKULIĆ, N., MARIĆ, S., GALAMBOS, L., RADOŠEVIĆ, D. & KRPO-ĆETKOVIĆ, J. (2013) New distribution data and population structure of the European mudminnow *Umbra krameri* in Serbia and Bosnia and Herzegovina. *Journal of Fish Biology*, 83, 659–666.

STERBETZ, I. (1963) Adatok a lápi póc (*Umbra krameri* WALBAUM) és a tarka géb (*Proterorhinus marmoratus* PALLAS) kárpát-medencei elterjedéséhez. *Vertebrata Hungarica*, 5, 15–18. [In Hungarian]

TAKÁCS, P., ERŐS, T., SPECZIÁR, A., SÁLY, P., VITÁL, Z. & FERINCZ, Á. (2015) Population genetic patterns of threatened European mudminnow (*Umbra krameri* Walbaum, 1792) in a fragmented landscape: implications for conservation management. *PLoS ONE*, 10(9), e0138640.

TATÁR, S. (2010) Seed longevity and germination characteristics of six fen plant species. *Acta Biologica Hungarica*, 61(Suppl.), 197–205.

TATÁR, S., SALLAI, Z., DEMÉNY, F., URBÁNYI, B., TÓTH, B. & MÜLLER, T. (2010) A lápi póc fajvédelmi mintaprogram. *Halászat*, 103, 70–75. [In Hungarian]

VALACHOVIČ, D. & KOVÁČ, V. (1998) Ochrana blatniaka tmavého ex situ v CHKO Záhorie. *Chránené územia Slovenska*, 35, 18–19. [In Slovak]

WANZENBÖCK, J. (1995) Current knowledge on the European mudminnow *Umbra krameri* Walbaum, 1792. *Annalen des Naturhistorischen Museums in Wien*, 97, 439–449.

WANZENBÖCK, J. & SPINDLER, T. (1995) Rediscovery of *Umbra krameri* WALBAUM, 1792, in Austria and subsequent investigations. *Annalen des Naturhistorischen Museums in Wien*, 97, 450–457.

WITZENBERGER, K.A. & HOCHKIRCH, A. (2011) *Ex situ* conservation genetics: a review of molecular studies on the genetic consequences of captive breeding programmes for endangered animal species. *Biodiversity and Conservation*, 20, 1843–1861.

ZAKĘŚ, Z., SZCZEPKOWSKI, M., PARTYKA, K. & WUNDERLICH, K. (2013) Effect of gonadotropin hormonal stimulation on out-of-season propagation success of different year classes of indoor-reared pikeperch (*Sander lucioperca* L.). *Aquaculture International*, 21, 801–810.

Biographical sketches

SÁNDOR TATÁR is a biologist whose main focus is on the vegetation, hydrobiology and restoration of wetlands. BÁLINT BAJOMI works on the reintroduction of threatened animal species. ANDRÁS SPECZIÁR studies the ecology and diversity of fish and chironomid assemblages. BALÁZS TÓTH works on in situ protection and monitoring of fish. MAGDOLNA MÜLLERNÉ TRENOVSZKI, BÉLA URBÁNYI and TAMÁS MÜLLER work on the ex situ conservation of protected and threatened fish species. BÉLA CSÁNYI and JÓZSEF SZEKERES monitor surface waters in light of the EU Water Framework Directive, and assess the status of their macroinvertebrate, faunal and fish assemblages.

TABLE 1 Fish assemblages and macrophyte coverage recorded during surveys at sites of existing or previously known populations of European mudminnow *Umbra krameri* in Hungary's Carpathian Basin (Fig. 1), with site number and survey date in parentheses.

	Still waters						Watercourses				
	Ócsa Landscape Protection Area (Site 3; 19 June 2008)	Pond Farmos (Site 5; 16 Nov. 2012)	Lake Báb (Site 7; 2 Apr. 2010)	Pócos Pond A (Site 8; 19 June 2008)	Pócos Pond B (Site 9; 29 June 2009)	Fen at South M0 Bridge (Site 11; 7 & 16 Sep. 2010)	Császárvíz Canal, upper section (Site 1; 26 June 2014)	Császárvíz Canal, lower section (Site 2; 16 Nov. 2012)	Felső-Tápió Stream (Site 4; 16 Nov. 2012)	Csaronda River (Site 6; 2 Apr. 2010)	Gőgő-Szenke Stream (Site 10; 2 Apr. 2010)
Abramis brama								1			
Ameiurus melas[2]									2		
Blicca bjoerkna									5		
Carassius carassius[1]		66									4
Carassius carassius × *Carassius gibelio*		3									
Carassius gibelio[2]		14					1	13	5		3
Cobitis elongatoides								88	32		
Gobio gobio								7			

Lepomis gibbosus[2]								1	2		
Misgurnus fossilis[1]		55			1			4	3	1	6
Perca fluviatilis								10			
Perccottus glenii[2]			12							8	
Proterorhinus marmoratus								20	1		
Pseudorasbora parva[2]							38	17	1		
Rhodeus sericeus								38	1		
Rutilus rutilus									1		2
Squalius cephalus								28			
Tinca tinca						9					
Umbra krameri[1]	12			27	22	21			2		19
Total no. of individuals	12	138	12	27	23	30	39	227	55	9	34
Total no. of species	1	4	1	1	2	2	2	11	11	2	5

% abundance of invasive species	0.0	10.1	100.0	0.0	0.0	0.0	100.0	13.7	18.2	88.9	8.8
% invasive species	0.0	25.0	100.0	0.0	0.0	0.0	100.0	27.3	36.4	50.0	20.0
% macrophyte coverage (characteristic species)*	60 (*Lemnetum minoris*)	100 (*Ceratophylletum demersi*)	100 (*Lemnetum minoris*, *Hydrocharis morsus-ranae*)	100 (*Lemnetum minoris*)	8 (*Lemnetum minoris*, *Lemna trisulca*)	100 (*Ceratophylletum demersi*, *Lemnetum minoris, Elodea canadensis*)	5 (*Lemnetum minoris*)	0	0	0	100 (*Lemnetum minoris*, *Lemna trisulca*)

[1]Protected/rare marshland fish species

[2]Invasive species

TABLE 2 Outcome for European mudminnow saved from three sites (Fig. 1), bred in the laboratory and released at three artificial ponds, with capture site, number of individuals captured, mortality during the acclimatization period, number of individuals bred in the laboratory, post-breeding mortality, number of individuals released after laboratory breeding, and release date and location. More than half of mortalities were attributable to poor condition, age or disease.

Capture site	No. of individuals captured (females, males)	Date of capture	No. of deaths during acclimatization	No. of individuals bred in laboratory	No. of deaths post breeding	No. of individuals released	Release date	Release site
Gőgő-Szenke stream	15 (8,7)	2 Apr. 2010	2	13	0	13	6 Apr. 2010	Illés Pond I
Pócos Pond B	6 (2,4)	6 Apr. 2010	2	4	0	4	31 May 2010	Illés Pond IV
Fen at the South M0 Bridge	21 (9,12)	7 & 16 Sep. 2010	12	9	6	3	7 June 2011	Illés Pond III

TABLE 3 Details of releases of European mudminnow and of captive bred and nursed stocks.

Origin of fish	Released offspring				
	Locality	Date	No. of individuals	Age (days)	Standard length (cm)
Gőgő-Szenke Stream	Gőgő-Szenke Stream	27 May 2010	100	39	1.5–2
	Illés Pond I	31 May 2010	103	43	1.5–2
	Illés Pond I	22 Sep. 2010	25	187	3–5
	Gőgő-Szenke Stream	12 Oct. 2010	50	208	3–6
Pócos Pond B	Illés Pond IV	31 May 2010	33	43	1.5–2
	Illés Pond IV	29 May 2012	371[1]	55	2
Fen at the South M0 Bridge	Illés Pond VI	14 Oct. 2011	20	188	3–5
	Illés Pond III	10 Aug. 2011	41	123	2–3
	Fen at the South M0 Bridge (Ráckeve Danube	10 Aug. 2011	114[2]	c. 131	2–3
	Branch Natura 2000 Site, Czuczor Island)	28 Sep. 2011	143[2]	c. 180	2–3
	Ráckeve Danube Branch Natura 2000 Site (Csupics Island)[3]	12 June 2012	121	429	5–7

[1]From laboratory breeding of 13 parent fish captured in Illés Pond IV on 21 March 2012 and re-released into the same pond on 21 April 2012

[2]Natural offspring of saved adults released to Illés Pond III

[3]Csupics Island is a nearby and similar natural habitat to the partially destructed fen at the South M0 Bridge (Czuczor Island).

TABLE 4 Results of monitoring in Illés Ponds (Fig. 1), with numbers of European mudminnow, crucian carp and weatherfish, mean recapture rate (no. recaptured relative to no. stocked) and mean coverage of macrophytes. As Illés Pond IV is connected to Pócos Pond B to provide a refugee for natural marsh fish assemblages in dry periods, recapture rate is not relevant. In some cases low water temperature probably decreased the sampling efficiency.

	Umbra krameri	*Carassius carassius*	*Misgurnus fossilis*	Mean % macrophyte coverage (taxa)
Illés Pond I (created July 2008; sampled 6 times during May 2010–Sep. 2012)				
No. of individuals stocked	150	50	50	
No. of one-summer-old natural offspring	14	47	0	
Mean recapture rate (%)	21	18	4	< 1 (*Utriculariavulgaris*)
Illés Pond III (created July 2009; sampled 7 times during Aug. 2011–Sep. 2012)				
No. of individuals stocked	48		200	
No. of one-summer-old natural offspring	550[1]		0[2]	
Mean recapture rate (%)[3]	8		0	80 (*Ceratophyllum demersum*)
Illés Pond IV (created July 2009; sampled 5 times during May 2010–Sep. 2012)				

	Umbra krameri	*Carassius carassius*	*Misgurnus fossilis*	Mean % macrophyte coverage (taxa)
No. of individuals stocked	407			
No. of one-summer-old natural offspring	5[4]	10[4]		
Mean recapture rate (%)	18			89 (*Chara* sp.)
Illés Pond VI (created Sep. 2010; sampled twice during June–Sep. 2012)				
No. of individuals stocked	20	108	20	
No. of one-summer-old natural offspring	0	0	0	
Mean recapture rate (%)[3]	20	78	35	80 (*Ceratophyllum demersum*)
Mean (range) of recapture rates for all ponds (%)	17 (8–21)	48 (18–78)	13 (0–35)	

[1]257 captured larvae of natural progeny were used to reinforce the threatened parental population (Table 3).
[2]Weatherfish larvae released at small size (c. 1 cm) were probably still smaller than the mesh size.
[3]On some occasions fish sampling was ineffective because of dense vegetation, and thus these results are incomplete.
[4]Illés Pond IV is connected to native Pócos Pond B, and therefore the exact place of spawning cannot be identified.