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Conservation of bullhead *Cottus perifretum* in the Demer River (Belgium) basin using re-introduction

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Summary

Until 2003 *Cottus perifretum* was believed to be extinct from the Demer River basin in Flanders, Belgium. However, that year a relict population of this bullhead species was found in the Dorpbronbeek. The Research Institute for Nature and Forest (INBO) and the Agency for Nature and Forests (ANB) launched a conservation project to preserve this population by re-introducing cultured progeny to the Zevenbronnenbeek. This headwater stream was carefully selected from seven evaluated potential locations within the Demer River basin based on water and habitat quality and food availability. In October 2008, 1220 cultured age 0+ bullhead were released. To enlarge the chance for success, ceramic roof tiles were added to the stream as artificial spawning substrates. In 2009, the success of the re-introduction was assessed. The recaptured fish had grown since their release and were in visual good condition, but most important, natural reproduction occurred.

Introduction

The bullhead is a small bottom-dwelling freshwater cottid, well adapted to the benthic life style (Lelek, 1987). In Belgium two indigenous bullhead species occur: *Cottus perifretum* in the Scheldt River drainage and *Cottus rhenanus* in the Meuse River drainage (Volckaert et al., 2002).

Bullhead are an indicator for the naturalness of streams (Tomlinson and Perrow, 2003). The species naturally occurs in unpolluted, cool, well-oxygenated streams with a moderate velocity. Furthermore, the presence of coarse hard substrates like stones is of vital importance to assure viable bullhead populations (Knaepkens et al., 2002). These substrates provide daytime shelter for bullhead and more importantly are used as spawning substrates (Smyly, 1957). Chemical water pollution, habitat deterioration and fragmentation are the major threats for bullhead (Vandelannoote et al., 1998).

In Flanders, the northern region of Belgium, *Cottus perifretum* historically occurred in large continuous populations in the river drainage of the river Scheldt. Today the bullhead is confined to some headwater streams where the environmental conditions are sufficient. Consequently, only very few, small, fragmented and isolated populations remain (Vandelannoote et al., 1998). Bullhead is listed as IUCN susceptible on the national Red List and is fully protected by law (Vandelannoote et al., 1998). It is also included in Annex II of the European Habitats Directive 92 ⁄ 43 ⁄ EEC.

A few small *Cottus perifretum* populations still occur in the Scheldt River drainage. In the Demer River, a tributary of the Scheldt River *Cottus perifretum* was considered to be extinct. Historical data show that in the past bullhead was common in this entire river basin (Vrielinck et al., 2003) but declined dramatically with the last observation in 1957 (Timmermans, 1957). Despite intensive fish surveys, no bullhead was recorded in this basin since. In 2003, in a water quality sample, the Flemish Environment Agency (VMM) accidentally caught a juvenile bullhead in the Dorpbronbeek, a small tributary of the Kleine Gete in the Demer River basin. Subsequent intensive monitoring showed that a small bullhead population still occurs in this water over a distance of about 200 m.

A genetic study using microsatellites revealed that the discovered population is a relict of *Cottus perifretum* in the Demer River basin (Horemans, 2006). The population is similar to the other bullhead populations in the Scheldt River drainage but has five unique alleles. Previous genetic research showed that the genetic diversity is low for *Cottus perifretum* and extremely fragmented between the different populations (Knapen et al., 2003). All populations can thus be considered as management units and should be managed separately. To prevent substantial losses of genetic variability in *Cottus perifretum* in Belgium, management should aim to protect and conserve as many of these populations as possible (Knaepkens, 2004).

Due to recent habitat deterioration and the small area, the newly found relict population in the Dorpbronbeek is seriously endangered. For this reason the Research Institute for Nature and Forest (INBO) immediately advised the authorities to take measures to safeguard the population *in situ* by restoring and protecting its habitat. Together with the Agency for Nature and Forests (ANB), INBO also launched a restoration project to conserve the unique gene pool of the Dorpbronbeek *ex situ* by re-introducing cultured progeny originating from the relict population to another suitable habitat in the Demer River basin. Preceding the actual re-introduction in 2008, a captive breeding program and habitat suitability study were carried out. In 2009 the first post-release monitoring was performed.

Material and methods

Study area

The Demer River basin has a catchment area of 1920 km^2. The Dorpbronbeek where the relict population was found, is a small tributary in the sub-basin of the Kleine Gete nearby Orsmaal.

Habitat suitability assessment

In 2007 INBO assessed the habitat suitability for bullhead of seven locations within the Demer River basin: the

Waarbeek-Deesbeek, a tributary of this stream, the Molenbeek, the Velpe (nearby Opvelp), the Schoorbroekbeek, a nameless tributary of the Kleine Gete (nearby Neerhespen) and the Zevenbronnenbeek. These sites were selected as potential re-introduction locations based on their good chemical and biological water quality. At each location, INBO evaluated the critical habitat features for bullhead at two randomly chosen sections. The assessed habitat features were derived from previous studies (Carter et al., 2004; Van Liefferinge et al., 2005) and other literature (Tomlinson and Perrow, 2003). The habitat in the Dorpbronbeek was used as a reference biotope because of its similarity to the other locations within the same basin.

The water depth, width of the stream and velocity at 5 cm above the streambed (Marsh-McBirney flowmeter model 201) were measured at randomly chosen points, the composition of the substrate, the channel structure (natural or regulated), the structure of the river banks and the amount of instream vegetation on the other hand were determined over the entire length of the assessed sections. Finally the locations were checked for possible migration barriers for bullhead. To determine the present fish community, the locations were sampled by electric fishing (portable model DEKA 3000). Fishing was carried out by wading a single time in upstream direction with one handheld anode with a net attached to it. The sampled fish were identified to species and counted. The food availability for bullhead was assessed based on data on the diversity and abundance of aquatic invertebrates from the Flemish Environment Agency (VMM).

Captive breeding

Captive breeding of bullhead from the relict population is carried out at the fish culture centre of INBO in Linkebeek (Belgium) since 2004.

During the winter brood fish are maintained (<40 kg bullhead per ha) in earthen ponds with high densities of *Gammarus* spp. and other aquatic invertebrates. Halved flower pots and ceramic roof tiles are distributed on the bottom (1 m^{-2}) as shelter for the fish. Each year before the end of February, the mature bullhead are collected from the ponds and placed in four outside raceways (length 100 cm, width 40 cm, depth 15 cm), with a maximum of 6–10 bullhead couples per raceway. The raceways are supplied with a continuous flow (15 L min^{-1}) of clean and well-oxygenated water. The bottom of the raceways is covered with coarse sand, stones, halved flower pots and ceramic tiles (5 m^{-2}) and perforated plates are used to cover the raceways. The bullhead are fed with live *Gammarus* spp. and exposed to natural water temperature and light cycles to induce spawning.

After spawning the eggs are left in the raceways attached to the artificial spawning substrates for the males to take care of them until hatching. After hatching, the larvae are flushed from the raceways and collected in a basin underneath the outlet. From there, they are transferred to grow-out ponds where they feed on natural zooplankton and aquatic invertebrates. Each pond is stocked with larvae of the same size to prevent cannibalism.

Re-introduction

In October 2008, 1220 cultured age 0+ bullhead were released at four locations in a 1600 m reach of the Zevenbronnenbeek, with release location four situated at the origin and location 1 located most downstream of the reach. After gradual acclimation, the fish were evenly distributed across the release sites (approximately one individual per m). To enhance the spawning habitat, ceramic roof tiles were added to the stream as artificial spawning substrates (Table 1). Before the release, the total length (mm) and weight (0.1 g) of 120 randomly selected fish were measured.

Post-release monitoring

In March, August and September 2009, the Zevenbronnenbeek was sampled. The method was similar to the electrofishing that was carried out during the habitat suitability assessment. The captured fish were identified to species, counted, measured and then returned to the water. In March a 100 m stretch at release location 1 was sampled, in August 30 m was fished at each release location and in September five 50 m stretches were sampled: three stretches (50 m) downstream of release site 1, one 50 m stretch between release sites 2 and 3 and one at release location 4.

From January until May 2009, the tiles were, at the end of each month, carefully inspected for the presence of bullhead and bullhead egg-clusters underneath them. If a bullhead was present, it was captured and the egg-clusters were counted. The sex of the captured fish was determined and its total length was measured (mm). Immediately afterwards, the tile and fish were carefully replaced in the original position.

Results

Habitat suitability assessment

The Zevenbronnenbeek and the Schoorbroekbeek were the only locations that were positively assessed based on their suitable habitat and sufficient food availability. Because of the slightly better water quality in the Zevenbronnenbeek, this stream was selected for the pilot re-introduction.

The Zevenbronnenbeek is a small (width: 1 m), shallow, moderate flowing headwater stream (Table 2) with a continuous length of about 3.3 km from its origin on. It has a natural, sinuous channel form with pool and riffle structures and a very diverse habitat. Mud is the dominant substrate but areas of gravel also exist. Natural hard substrates like stones and woody debris are found along the entire streambed. *Gammarus* spp., chironomids and several oligochaets are found in large numbers. During electrofishing only three-spined stickleback (*Gasterosteus aculeatus*) were captured.

Captive breeding

Breeding bullhead, *Cottus perifretum* as described earlier is successful. Immediately after the transfer to the raceways, the male bullhead started to make nests underneath the artificial

Table 1
The length (m) and the number of ceramic tiles added to the streambed at the four release locations

Location	Length (m)	Number of ceramic tiles added
1	150	15
2	250	16
3	450	20
4	300	17

Table 2
The measured habitat features at two randomly chosen sections in the Zevenbronnenbeek

	Section 1	Section 2
Average depth (range)	9.4 cm (6–11 cm)	15 cm (10–19 cm)
Average velocity (range)	20.4 cm s^{-1} (14–29 cm s^{-1})	12.4 cm s^{-1} (6–20 cm s^{-1})
Width	1 m	1 m
Substrate composition	Mainly mud + gravel areas	Mainly mud + woody debris
Channel structure	Natural, sinuous with pool-riffle	Natural, sinuous with pool-riffle
Migration barriers	None	None
Instream vegetation cover	0–90%	0–10%
Structure of the river banks	No hollow river banks	No hollow river banks
Fish community	Three-spined stickleback	–

substrates. By the end of March, when the water temperature reached 12°C, natural spawning occurred. No hormonal induction had to be used.

The eggs were deposited as clusters on the ceiling of the artificial substrates where the males took care of them until hatching. Artificial incubation of spawned eggs in incubation bottles was not that successful in the past. When the hatched larvae absorbed their yolk sac, they had to be fed. Since intensive rearing in tanks or aquaria on live and, later on, on dry food was not really successful until now, the bullhead were raised in grow-out ponds. In 2008 in total 3237 bullhead larvae were collected from the raceways and stocked into four grow-out ponds. In October in total 1321 age 0+ bullhead (41%) were harvested. Survival of bullhead larvae in the ponds ranged from 16 to 69%.

Post-release monitoring

During electrofishing in March, August and September 2009, only bullhead and three-spined stickleback (*Gasterosteus aculeatus*) were captured on and close to the release locations. More downstream of release location 1, no bullhead were captured at all (Table 3).

In late summer two sizes of bullhead were sampled. The length-frequency distribution of the bullhead sampled in August and September (Fig. 1) reveals the presence of 2 year classes: age 0+ bullhead and age 1+ bullhead. Based on this graph and on literature (Smyly, 1957; Mills and Mann, 1983), we considered bullhead smaller than 5.5 cm young-of-the-year bullhead. In total 30.5% of the bullhead sampled in August and September were age 0+ fish. The captured age 0+ bullhead were in late summer still smaller compared to the cultured 0+ fish that were released in October 2008 (Fig. 1). The recaptured bullhead, however, had increased in size since their release (Fig. 1). In October 2008 the total length of the released fish averaged 5.5 ± 0.9 cm (range 3.1–8.0 cm) and the weight 1.9 ± 1.2 g (range 0.2–6.9 g), in late summer 2009 the bullhead averaged 8.7 ± 1.6 cm (range 6–11.6 cm).

The number of captured adult and juvenile bullhead differed between the release locations (Table 3). At release location 4 more adult bullhead were captured compared to the other locations but almost no juveniles. At the three other releasing sites both juveniles and adults were sampled. Most juveniles were captured at location 2.

At the end of January 2009, no territorial bullhead were observed underneath the tiles (Table 4). By the end of February the first sexually active males were detected. Their number increased from February with a peak in March and decreased again afterwards (Table 4). The first egg-clusters were observed in March, altogether 18 egg-clusters were found

Table 3
The numbers, average total length (TL) and range of TL of adult and juvenile bullhead captured by electrofishing in March, August and September 2009 at the four release locations (RL), downstream of RL1 and at a stretch between release location 2 and 3 (RL2–RL3)

	Downstream RL1	RL1	RL2	RL2–RL3	RL3	RL4
March						
Adult						
Number	ns	9	ns	ns	ns	ns
Average TL	ns	6.5 ± 1.6	ns	ns	ns	ns
Range TL	ns	4.3–9.1	ns	ns	ns	ns
YOY						
Number	ns	0	ns	ns	ns	ns
Average TL	ns	–	ns	ns	ns	ns
Range TL	ns	–	ns	ns	ns	ns
August						
Adult						
Number	ns	2	3	ns	5	15
Average TL	ns	9.0 ± 1.9	9.5 ± 1.9	ns	9.5 ± 1.9	8.5 ± 1.4
Range TL	ns	8.8–9.1	7.9–11.6	ns	7.4–11.3	6.8–11.2
YOY						
Number	ns	1	7	ns	2	0
Average TL	ns	4.6	4 ± 0.3	ns	3.9 ± 0.4	–
Range TL	ns	–	3.7–4.4	ns	3.6–4.1	–
September						
Adult						
Number	0	ns	ns	11	ns	14
Average TL	–	ns	ns	8.4 ± 1.8	ns	8.6 ± 1.6
Range TL	–	ns	ns	6.0–11.2	ns	6.1–10.8
YOY						
Number	0	ns	ns	11	ns	1
Average TL	–	ns	ns	4.5 ± 0.5	ns	4.2
Range TL	–	ns	ns	3.8–5.4	ns	–

ns, Means no sampling was performed at that location.

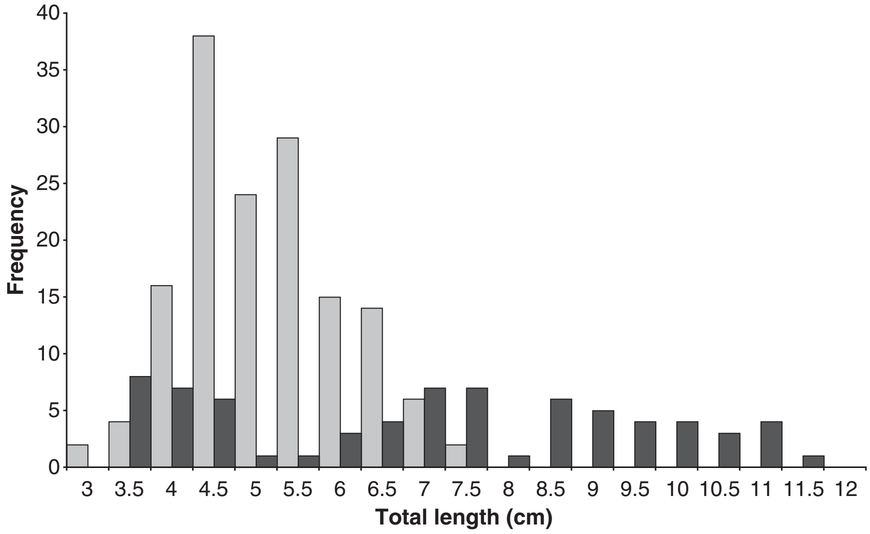

Fig. 1. The length-frequency distribution of 150 released bullhead in October 2008 (grey bars) and of the bullhead sampled by electrofishing in the Zevenbronnenbeek in August and September 2009 (black bars)

Table 4
The numbers of territorial males, females and egg clusters found underneath the artificial substrates during observations in spring 2009

	Number of males	Number of females	Number of egg clusters
January	0	0	0
February	6	0	0
March	24	2	10
April	11	1	8

attached to the tiles. One extra egg-cluster was seen attached to a submerged hollow root.

Discussion

Habitat suitability assessment

The release site should be carefully selected since a re-introduction is more likely to succeed in a suitable habitat. The selected site must provide habitat coverage that encompasses all the life stages of bullhead, e.g. spawning, juvenile and adult habitat (Cowx and Harvey, 2004).

For this reason, the Zevenbronnenbeek was selected. This tributary is located within the Demer River basin, where *Cottus perifretum* was common in the past (Vrielinck et al., 2003). It is even situated in the same sub-basin as the Dorpbronbeek. Electrofishing made clear that no undetected remnant bullhead population occurs.

The chemical and biological water quality at the Zevenbronnenbeek is high. A good water quality seems to be the most important prerequisite for bullhead. Although it appears to be more tolerant to pollution as previously thought (Utzinger et al., 1998), they are sensitive to organic pollution due to the lower oxygen saturation levels this can cause (Tomlinson and Perrow, 2003). Bullhead require dissolved oxygen concentration of above 80% saturation. The BOD should always be lower than 3 mg O_2 L^{-1}.

Besides good water quality, water depth, velocity above the streambed and the substrate composition are the key habitat features for bullhead (Tomlinson and Perrow, 2003). Based on our habitat suitability assessment we can conclude that the habitat of the Zevenbronnenbeek satisfies all requirements.

Bullhead prefer shallow streams with depths between 20 and 50 cm. The average depth in the shallowest assessed part of the Zevenbronnenbeek is only 9.4 cm with a measured minimum of 6 cm and a maximum of 11 cm. However Utzinger et al. (1998) state that water depth is not critical for bullhead, providing it is >5 cm and the flow is adequate to prevent warming of the water and oxygen depletion.

Bullhead need a variety of stream velocities during their life time between 0.2 and 1.0 m s^{-1}, so juveniles as well as adults can find their optimal habitat (Seeuws et al.,1999). Only in natural sinuous streams with pool and riffle structures these large variations can be found close to each other. A velocity below 10 cm s^{-1} is considered unsuitable. The water velocities measured at the Zevenbronnenbeek lie within this range.

The presence of a coarse, hard substrate of clean gravel and stones is necessary for bullhead to complete their reproductive cycle (Smyly, 1957). In the Zevenbronnenbeek this can be a critical factor, the dominant substrate being mud. However, the same situation was observed at the reference biotope in the Dorpbronbeek and still a self-sustaining population occurs. If necessary (due to lack of sufficient stones) also macrophytes and woody debris can be used as shelter and spawning substrate. To enlarge the chance for success of the re-introduction, the habitat can also be enhanced by adding artificial substrates like ceramic roof tiles and flower pots to the stream. This has already been done for bullhead in Flanders (Knaepkens et al., 2004).

Because of its natural sinuous form, the habitat of the Zevenbronnenbeek is highly diverse and suitable for bullhead. Tomlinson and Perrow (2003) mentioned that a natural, sinuous channel form with associated riffle and pool structure provides the necessary substrates and velocities.

The diet of bullhead consists of all kinds of different benthic invertebrates. In the Zevenbronnenbeek *Gammarus* spp. chironomids and several oligochaets are abundant. These are excellent food for bullhead (Hyslop, 1982). No competitors like stone loach or large predatory fish like brown trout occurred.

Captive breeding

The lack of stocking material is often a major constraint in a re-introduction program (Cowx, 1998). Therefore a captive breeding program was developed for *Cottus perifretum* from the Demer River basin.

At the facility of INBO the captive breeding of bullhead was done in semi-natural conditions. In early spring mature bullhead were transferred to breeding systems designed to mimic their natural breeding habitat. In this way, natural spawning could easily be induced without hormonal stimulation by exposing the brood fish to natural light and temperature. From the little information we have from literature, also the bullhead species *Cottus gobio* was already bred in a quite similar way (Bohl and Ferling, 1994). In captivity spawning started in March when water temperature reached 12°C, just like in nature (Smyly, 1957).

The presence of coarse, hard substrates is essential for bullhead to complete their reproduction (Knaepkens et al., 2002) since bullhead females attach their egg-clusters to the underside of this substrate (Smyly,1957). Therefore we provided roof tiles and halved flower pots to the breeding systems. They make excellent artificial spawning substrates in breeding systems as well as in natural streams (Bohl and Ferling, 1994; Knaepkens et al., 2004). The substrates also provide shelter for the fish which in turn can prevent aggressive behaviour and hence lower stress among the fish.

Artificial incubation of bullhead eggs was far less successful compared to natural incubation with parental care of the male. Bohl and Ferling (1994) also observed this.

Although, these authors succeeded to rear the hatched larvae intensively on *Artemia* nauplii and frozen food, our attempts failed. Consequently, we tried to grow out the larvae in ponds. This alternative method proved to be successful, easy and less labour intensive.

Post-release monitoring

The recapture of bullhead nearby and on the release locations in the Zevenbronnenbeek, confirms that the habitat is suitable for bullhead. Although the species is known to recolonise fast, no bullhead were captured further downstream of release location 1. Most probably some restoration measures need to be put in place to improve water and habitat quality before we can expect bullhead to settle in this part of the Zevenbronnenbeek.

The recaptured bullhead were at age 1+ bigger than what was previously reported in Maitland and Campbell (1992) and in Seeuws et al. (1999). In spring 2009, natural reproduction already occurred. Apparently some of the released bullhead matured after 1 year. The growth rate of bullhead and therefore sexual maturity is highly dependent on environmental conditions (Tomlinson and Perrow, 2003). In lowland rivers with a high productivity bullhead are also known to grow fast and mature after 1 year (Mills and Mann, 1983). Also the culture conditions resulted in rapid growth and early sexual maturity.

The first sexually mature males were detected at the end of February underneath some roof tiles. Probably these males started to make their nests underneath the spawning substrates to attract a female (Tomlinson and Perrow, 2003). From March on, more males were seen underneath the tiles and at this time also egg-clusters were found. At the end of April again less males and egg-clusters were observed, implying that the spawning season was at its end. This is similar to what is described in literature (Smyly, 1957) and to what was observed during breeding in captivity.

The dominant substrate in the Zevenbronnenbeek is mud. Although natural spawning substrates like stones and woody debris also occurred along the stream, we decided to enhance the habitat by adding tiles to the stream in order to enlarge the chance for spawning success. The use of the tiles by the bullhead confirms that this is a promising, easy and cheap tool for the conservation of bullhead populations (Knaepkens et al., 2004). Also the present natural substrates were used as nests.

Natural recruitment was successful since young-of-the-year bullhead were sampled in late summer. The difference in captured numbers of juvenile and adult bullhead reflects the diversity of the habitat along the stream. Location 2 seems to be an excellent nursery habitat for young bullhead with its shallow riffles and submerged vegetation. Location 4 on the other hand seems more suitable for adult bullhead.

To achieve a favourable conservation status more than 40% of the individuals should be in the 0+ age class (Cowx and Harvey, 2004). In the Zevenbronnenbeek only 30.5% of the captured bullhead in late summer were young-of-the-year. However, care must be taken when interpreting this data. Not only can the life histories and reproductive strategies of bullhead vary strongly between different river systems (Cowx and Harvey, 2004), the distinction between age 0+ and 1+ bullhead was also made based on literature data (size of 5.5 cm). Most likely, some of the young-of-the-year bullhead were larger due to their fast growth and thus not recognised as young-of-the-year. Furthermore, when we exclude the data of release location 4, where almost no juveniles were sampled, 50% of the bullhead captured at the other locations belong to the 0+ age class.

Anyhow, further yearly monitoring is recommended to see if a healthy, self-sustaining population can establish in the Zevenbronnenbeek because in a bullhead population with fast growth and early maturation an annual successful recruitment is necessary in order to maintain the population size (Mills and Mann, 1983). Also the influence of inbreeding on the genetic variability, the dispersion of the bullhead and the longevity of the artificial substrates should be followed on the longer term.

Conflict of interests

The authors declare no potential conflict of interests, neither have they received speakers fees, etc. from any commercial body within the past 2 years.

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