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Supporting natural populations of European bullhead (*Cottus gobio*) through *ex situ* breeding and rearing

A botos kölönte (*Cottus gobio*) természetes populációinak támogatása *ex situ* szaporítással és neveléssel

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ABSTRACT

Ex situ breeding and larvae rearing offer a promising tool for supporting declining natural populations of European bullhead (*Cottus gobio*), a small benthic fish of conservation concern. This study evaluated the success of captive reproduction and early larval rearing under controlled conditions as a preparatory step for future restocking efforts. Adult bullheads were maintained in aquarium settings, with spawning occurring between April 2 and 3, 2025, at temperatures of 9–13 °C. Six egg clutches, averaging 400 eggs each, were monitored; larvae commenced feeding 15–16 days post-hatching. Over a 3-week rearing experiment, three feeding treatments (fresh *Artemia*, frozen *Artemia*, and a mixed diet) were tested for effects on larval growth. Mortality was minimal in all groups; however, larvae fed exclusively on frozen *Artemia* exhibited significantly smaller body lengths and greater variability compared to those on fresh or mixed diets, which did not differ significantly. 751 feeding larvae were obtained from ~4,400 eggs, resulting in a survival efficiency of 17.1%. Unlike many studies lacking quantitative tracking from egg to juvenile stages, our results confirm the viability of captive breeding for *C. gobio* and provide essential baseline data for future conservation-driven propagation and release programs.

Keywords: spawning substrate, conservation biology, *Artemia* nauplii, larvae rearing

ÖSSZEFOGLALÁS

Az *ex situ* szaporítás és a lárvanevelés ígéretes eszközt jelent a csökkenő természetes állományok támogatására a botos kölönte (*Cottus gobio*) esetében, amely egy természetvédelmi szempontból jelentős, kis termetű, fenéklakó halfaj. A jelen tanulmány az *ex situ* szaporítás és a korai lárvanevelés sikerességét értékelte ellenőrzött körülmények között, mint előkészítő lépést a jövőbeli visszatelepítési programokhoz. Az ivarérett egyedeket akváriumi környezetben tartottuk, az ívás 2025. április 2–3. között zajlott, 9–13 °C közötti hőmérsékleten. Hat ikratétel, egyenként átlagosan 400 ikrával, került megfigyelésre; a lárvák a kikelést követően 15–16 nappal kezdték meg a táplálkozást. Egy 3 hetes nevelési kísérlet során három etetési kezelést (élő *Artemia*, fagyasztott *Artemia* és vegyes étrend) vizsgáltunk a lárvák növekedésére gyakorolt hatás szempontjából. A mortalitás minden csoportban minimális volt; ugyanakkor a kizárólag fagyasztott Artemiával etetett lárvák szignifikánsan kisebb testhosszal és nagyobb méretváltozatossággal rendelkeztek, mint az élő vagy vegyes étrenden nevelt egyedek, amelyek között nem mutatkozott szignifikáns különbség. Összesen 751 táplálkozó lárvát sikerült előállítani ~4.400 ikrából, ami 17,1%-os túlélési hatékonyságnak felel meg. Ellentétben számos olyan vizsgálattal, amelyben nem követték kvantitatívan a fejlődést az ikraállapottól a juvenilis korig, eredményeink megerősítik az *ex situ* tenyésztés életképességét *C. gobio* esetében, és alapvető kiinduló adatokat szolgáltatnak a jövőbeni, természetvédelmi célú szaporítási és kitelepítési programokhoz.

Kulcsszavak: ívóaljzat, természetvédelmi biológia, *Artemia* naupliusz, lárvanevelés

INTRODUCTION

Running water ecosystems face increasing global threats from urbanisation, industrialisation, agriculture, and climate change, as human population growth intensifies habitat alteration, pollution, and water exploitation, making integrated management and conservation efforts increasingly urgent (Malmqvist and Rundle, 2002). In this context, protecting cold-water rheophilic fauna is particularly critical, as rising water temperatures disrupt their physiological and ecological balance, threatening their survival and distribution and underscoring the need for targeted research and conservation measures (Bonacina et al., 2022). Furthermore, translating scientific research into practical conservation action is essential for developing evidence-based approaches that effectively strengthen the protection and sustainable management of freshwater biodiversity (Bylak and Kukuła, 2020). The European bullhead (*Cottus gobio*) is a widely distributed species in Europe and inhabits a very specific environment characterised by clear, fast-flowing, hard-bottomed, and oxygen-rich riverine habitats, which are increasingly fragmented and threatened across its range (Tomlinson and Perrow, 2003; Imecs and Nagy, 2015). Due to habitat degradation and population declines, *ex situ* breeding and reintroduction programs have become essential tools for conserving this species and restoring its populations in affected areas (Zanetti et al., 2014; Imecs et al., 2015; Liefferinge et al., 2021). In the European Union, significant efforts are being made to protect the species and its habitat (e.g., LIFE07 NAT/IT/000433, 2009–2014, Italy; current project SMIS Code 151922, 2022–2025, Romania). *C. gobio* is listed in Annex II of the Habitats Directive (Council Directive 92/43/EEC), forming the basis for Natura 2000 special conservation areas. In this context, management plans are being developed to implement conservation measures that promote inclusive and participatory environmental governance, ensuring the long-term preservation of species through integrated, multi-stakeholder approaches (Stringer and Paavola, 2013). The effectiveness of Natura 2000 sites, however, depends on the translation of these plans into coordinated, basin-scale actions that can reduce anthropogenic pressures and deliver measurable outcomes for freshwater biodiversity conservation (Gavioli et al., 2023). Scientific research contributes by providing evidence-based knowledge that guides the design, implementation, and evaluation of effective management measures for fish conservation (Boon and Baxter, 2020).

This is also the case for the Cheile Bicazului – Hășmaș National Park and Natura 2000 sites (Transylvania, Romania), where, according to their integrated management plan, the species was found only in the lower, isolated sections of the Oaia, Vereșceu and Licaș streams, flowing into Red Lake (Lacul Roșu), with a population density of merely 1.12 individuals per 100 m^2. We conducted the data collection for the plan between 2014 and 2015 and indicated that breeding and reintroduction of the species are essential conditions for the conservation of the population (Imecs & Nagy, 2015). According to the baseline data collected for the management plan, the species has an isolated habitat due to fragmentation at the target site (500–700 m above the Red Lake), supporting a population of approximately 40–50 individuals. Based on the study, the conservation status of the species is classified as "unfavourable–bad"

/ U2 (Imecs and Nagy, 2015). Consequently, the "good" conservation status listed in the site's data sheet has been downgraded to "moderate or declining," which now necessitates the formulation and implementation of conservation measures.

Conservation efforts for freshwater fish species in the region have included successful *ex situ* breeding and reintroduction programs, as demonstrated by Ford (IUCN, 2023) with the European mudminnow (*Umbra krameri*) or the endemic warm water rudd (*Scardinius racovitzai*). These programs highlight the importance of developing species-specific breeding and larval rearing protocols to maintain genetic diversity and enhance population viability. Such approaches have been critical in restoring populations and improving habitat conditions for endangered species in both Hungary and Romania (Imecs et al., 2015; IUCN, 2023, 2024).

The reproductive biology of *C. gobio* is notable for its low fecundity, with females laying only 121 to 357 eggs beneath stones during early spring (Mills and Mann, 1983). Others estimate that the average number of eggs per egg batch ranges between 200 and 600 (Piccinini et al., 2012) or 638 in a river and 272 in a brook (Marconato et al., 1993). Male bullheads provide parental care by guarding and aerating the eggs, often losing a significant portion of their body weight in the process, which can negatively impact larval survival (Marconato et al., 1993). Successful *ex situ* rearing requires careful attention to environmental conditions and feeding regimes (Piccinini et al., 2012).

This study aims to compare the larval growth of the European bullhead under three different feeding strategies, providing valuable insights for optimising rearing protocols in *ex situ* conservation programs. Improving larval survival and growth through tailored feeding strategies is critical for enhancing the success of breeding and reintroduction efforts, thereby supporting the long-term conservation of this species (Piccinini et al., 2012; Imecs and Nagy, 2015; Liefferinge et al., 2021).

The primary objective of this manuscript is twofold: (1) to develop and optimize breeding methodologies for the European bullhead under controlled *ex situ* conditions, including induced spawning and optimization of larval rearing in laboratory environments; and (2) to evaluate and facilitate the reintroduction of captive-bred individuals into newly established or restored habitats that offer ecologically suitable conditions for the long-term persistence of the species.

METHODS

The stocks involved in the experiment and their management are shown in Figure 1.

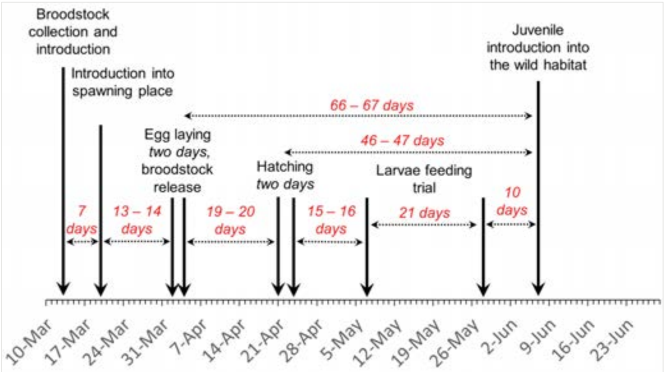


Figure 1. Flow diagram of fish handling during the experimental period

Broodstock

The specimens were captured using a Samus 725 MP electrofishing device (Samus Tech Ltd., Scientific Fishing Authorisation No. 04/03.02.2025) to sample the stream section located upstream of its confluence with Red Lake (Lacul Roșu; lat. 46.781374°, long. 25.785268°). Fish were placed in two cylindrical tanks (diameter: 120 cm; volume: 300 L). Each tank contained a spawning substrate consisting of a 24.5 × 22.5 cm stove tile with an inner rim (18 × 18 cm; height: 3 cm, Romanceram). Two openings were cut into the rim on opposite sides, and a hole was drilled in the upper surface to enhance water circulation. Initially, females and males were kept separately, after which they were introduced into mixed groups in two tanks, with the number of spawning substrates in each tank corresponding to the number of males present.

Twenty percent of the water in each tank was replaced every two days. The circular tanks were equipped with external filters (Aqua Zonic® AquaPRO 1800 L/h) to ensure continuous water circulation, simulating conditions suitable for a rheophilic species. During this period, the water temperature fluctuated between 6 and 9 °C (n = 7, 7.54 ± 1.14 °C), as in aquarium conditions, gradual temperature increase can initiate spawning earlier, giving the juveniles a 1-2 month advantage over those in the wild, thus increasing their chances of survival (Kopeika et al., 2008).

Reproduction, embryo and non-feeding larval development

The broodstock successfully spawned naturally 20–21 days after stocking (in both separate and mixed groups). Eggs together with the spawning substrate from the tanks were collected and incubated in five aquariums (V=40 L). These aquariums were equipped with an external filter (Aqua Zonic® AquaPRO 1800 L/h) similar to those in the tanks, as well as an internal filter. The internal filter was a 10 cm thick sponge adhered to the interior wall of the aquarium in such a way that the external filter draws water from the compartment behind the sponge, where only water was present. The filtered water was then returned through a pipe that passes through the sponge, thereby maintaining a continuous and stable circulation within the aquarium.

Water in the aquariums was replaced by 20% every two days.

Culture facilities used for the feeding experiment

The trial was conducted at the 'European Bullhead Breeding Centre', Băile Tuşnad (Transylvania, Romania) from May 7 to May 28, 2025. Larvae were reared for 21 days in a special recirculation larva rearing system. The larvae were kept in Petri dishes (Ø=13 cm, V=199 mL) with a fish escape netting (mesh size 1 mm) attached to the outer shell at a height of 2 cm. Water flow in each dish was maintained at 200 mL hour-1 (water changing rate was 5 times / Petri dish/hour). The recirculating system contained 100 L of water, which was filtered through an external filter (Aqua Zonic® AquaPRO 1800 L/h). Water movement was maintained by a circulation pump (Aquael® Circulator 1500). Approximately 30% of the total water volume was replaced daily. A total number of 90 larvae were stocked in 9 plastic tanks (30 fish / Petri dish, stocking density = 151 larvae/L). Fish were divided into three experimental groups (Table 1).

Table 1. Applied foods and feeding protocols tested in the experiments

	Replications	Feeding protocol (The fish were fed every 12 hours)	Abbreviation of the feeding protocol
Group 1	3	Only freshly hatched *Artemia* nauplii	Fresh
Group 2	3	At first, only freshly hatched *Artemia* nauplii; then, after a seven-day transition (freshly hatched in the morning, frozen in the evening), finally exclusively frozen *Artemia* nauplii	Mixed
Group 3	3	Only frozen *Artemia* nauplii	Frozen

Water quality management

Temperature was monitored once a day at 06.00 hours. Water chemistry parameters (Table 2) were measured using a Hanna (HI-9813-51) multiparameter and a Photometer PF-12 (Macherey-Nagel, Germany) portable analyser. The following water chemistry parameters were measured every third day: pH, electrical conductivity (EC, mS/cm), total dissolved solids (TDS, mg/L), temperature (T, °C), ammonium-ion (NH_4^+), nitrite-ion (NO_2^-), nitrate-ion (NO_3^-), phosphate-ion (PO_4^{3-}), general hardness (GH) and carbonate hardness/alkalinity (KH). The measurements were conducted throughout the entire experiment, including during the spawning and incubation phases.

Data collection

Fish were photographed (Kodak PIXPRO WPZ2) from a pre-installed stand, consistently at the same height. A ruler was placed beneath the Petri dishes so that the scale on the ruler could serve as a reference for measuring the fish sizes in subsequent photographs.

Table 2. Water parameters (mean ± SD) during the experiments

Parameter	Mean ± Standard Deviation
pH	8.24 ± 0.20
Electrical Conductivity (EC) *(mS/cm)*	0.659 ± 0.095
Total Dissolved Solids (TDS) *(mg/L)*	375.75 ± 53.59
Temperature (T) *(°C)*	13.5 ± 1.74
Ammonium-ion (NH_4^+) *(mg/L)*	1.35 ± 0.56
Nitrite-ion (NO_2^-) *(mg/L)*	0.068 ± 0.048
Nitrate-ion (NO_3^-) *(mg/L)*	11.18 ± 0.58
Phosphate-ion (PO_4^{3-}) *(mg/L)*	1.89 ± 0.48
Carbonate Hardness (KH / CH) *(°dKH)*	22.5 ± 1.77
General Hardness (GH) *(°dKH)*	25.38 ± 2.50

Data analysis

Every week, the total body length of all fish in each group was measured and recorded by using digital photos and Image J software (1.54d, Wayne Rasband, National Institutes of Health, USA). Given the high conservation importance of the larvae, we did not anaesthetise them or measure their body weight. The following parameters were calculated:

Daily growth length (DGL) = final total length – initial total length / 21 (mm/day)

Survival rate (%) = (number of live fish/number of fish used) × 100, and the data were analysed using the Chi-square test. Statistical analyses were carried out with SPSS for Windows version 21.0 (IBM SPSS, Armonk, NY: IBM Corp.). The results are expressed as means ± standard deviations. Normality and homogeneity of variance were subsequently assessed. Body length data were analysed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons.

RESULTS

Broodstock

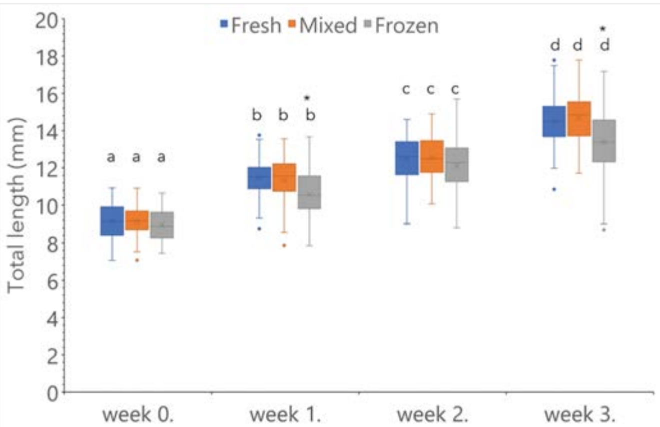
A total of 13 European bullhead (*Cottus gobio*) adult individuals were captured at a water temperature of 5 °C (March 13, 2025): 6 females (BW = 15.36±5.14 g, TL = 11.22±1.72 cm) and 7 males (BW = 14.41±2.28 g, TL = 10.93±0.83 cm). The experimental setup consisted of modified ceramic tiles that provided suitable spawning substrate and conditions for successful spawning and fertilisation (Imecs et al., 2024). After one week of separate maintenance, all fish (n = 13) were successfully introduced into mixed groups. In tank I, three males (BW = 12.5±1.9 g, TL = 10.6±1.0 cm) and three females (BW = 15.8±5.3 g, TL = 11.4±1.8 cm) were placed, while tank II contained four males (BW = 15.9±1.1 g, TL = 11.2±0.7 cm) and three females (BW = 14.9±6.1 g, TL = 11.0±2.0 cm). In both tanks, the number of spawning substrates corresponded to the number of males, and all individuals displayed normal activity and stable condition during the experimental period.

Captive breeding

Egg clutches appeared between April 2 and 3, 2025, 20-21 days after stocking. During this period, the temperature fluctuated between 9 and 13 °C (10.1±0.68 °C). Larval hatching commenced after a 19–20-day incubation period, faster than in the findings reported by Dorier (1942), who described larval development as a prolonged process, with hatching occurring after 25 days at 11 °C. This difference is likely due to the gradual temperature increase observed in our experiment, reaching up to 13 °C. The larvae began feeding after 15–16 days (Figure 1), during which time the water temperature in the aquariums ranged between 12 and 14 °C (11.71±1.45°C). After laying eggs, adult individuals were safely returned to their original habitat (April 2 and 3, 2025).

Larvae rearing

The basic data of the experiment are presented in Table 3. During the experiment, mortality among the treated groups was limited to only one individual each in the Mixed and Frozen groups. A growth plateau was observed between the first and second weeks in the Mixed and Fresh groups. The reasons for this are unknown. By the end of the third week, the growth rate increased again. This pattern was not observed in the group fed exclusively with frozen feed. Regarding the achieved body lengths, only the group fed exclusively with frozen *Artemia* showed statistically significantly lower average values compared to the other two groups, and they also exhibited the greatest within-group variability (Table 3, Figure 2). There was no statistically significant difference in body length between the other two groups (Fresh and Mixed, *P*>0.05).



Different lowercase letters indicate significant differences between diet groups within the same week based on Tukey's post hoc test (P < 0.05). Asterisks indicate significant differences within the same diet group compared to week 0 (*P* < 0.05).

Figure 2. Larval growth (total length, mm) of *Cottus gobio* over 21 days under three diet treatments

DISCUSSION

Captive breeding

All the fish involved in the experiment reproduced successfully (100%, 6 egg clutches / 6 spawning females). Considering the results of other research groups (Piccinini et al., 2012), this outcome can be regarded as excellent. The spawning tank and the water flow/environmental parameters we used—including the selection of the spawning substrate—were chosen based on two years of continuous testing results (Imecs et al., 2023, 2024). Our research group experimented with an *in situ* propagation method by installing a spawning cage at the natural breeding site, using commercially available hollow block bricks as spawning substrates (Imecs et al., 2025). This *in situ* method has previously been successfully applied to several other fish species, where both spawning and the early larval development were achieved with good results. For example, the endangered European mudminnow (*Umbra krameri*) was successfully bred, and the larvae were reared using a spawning substrate placed inside the cage (Müller et al., 2015). Through hormonally induced spawning, the thermal dwarf wild carp (*Cyprinus carpio carpio morpha hungaricus*) originating from Lake Hévíz (Hungary) was also successfully reproduced (Müller et al., 2024). However, in this species, due to flooding of the streams characteristic of its spawning habitat and the accumulation of suspended fine silt particles, the embryos in the egg clutch resulting from successful spawning were destroyed. Therefore, the use of this method is not recommended. However, without a spawning cage, the block bricks might be suitable as a spawning substrate for bullhead mating (Imecs et al., 2025).

Table 3. Summarized data about the experimental groups

Feeding protocol	TL_0 (mm)	TL_t (mm)	DGL (mm day^{-1})	S (%)
Fresh	9.1 ± 1.1	14.5 ± 1.3	0.26 ± 0.01	100
Mixed	9.1 ± 0.9	14.7 ± 1.3	0.27 ± 0.04	98.9
Frozen	8.9 ± 1.0	13.4 ± 1.8	0.21 ± 0.05	98.9

Note: TL_0 = initial total length; TL_t = total length at time t; DGL = daily growth in length; S = survival rate

Larvae rearing

It is important to highlight that, taking into account the hatching and feeding of the larvae, we were unable to establish a uniform initial stock. The approximate number of eggs per female, based on literature data, ranges from 121 to 357 eggs (Mills and Mann, 1983), between 200 and 600 eggs per egg clutch (Piccinini et al., 2012) or 638 in a river and 272 in a brook (Marconato et al., 1993). We estimate ~400 eggs/egg clutches (nonpublished own investigations), to which the fertilization and hatching rates must also be added. An important part of egg care is that eggs, along with the spawning substrate, were collected from the tanks and incubated separately in aquariums to prevent the caring male from consuming up to one-fifth of the eggs he tends (Marconato et al., 1993). Furthermore, we considered it important to start the experiment with larvae selected from mixed egg clutches originating from multiple females, to avoid the average species characteristics being overshadowed by the unique genotypic traits of individual fish. This is consistent with what is reported in the scientific literature, which indicates that individuals in England may spawn naturally with the local population up to four times in highly productive streams following natural conditioning, thereby enhancing the genetic background of the offspring, as different mating pairs are likely to form in each spawning event (Fox, 1978). However, other sources from the French Alps indicate that the species produces only a single clutch of eggs per year (Abdoli et al., 2005). This is likely related to habitat quality, population density, altitude, and the age structure of the population, which warrants further investigation.

During the experiment, the growth of the group fed exclusively with frozen *Artemia* lagged behind the other two groups (Fresh and Mixed). Several studies have shown that frozen *Artemia* nauplii often result in reduced larval growth compared with live *Artemia* in different fish species in different fish species, for example, *Lophiosilurus alexandri* (Nascimento et al., 2020) and *Acipenser fulvescens* (Valentin et al., 2017). The poorer growth performance was attributed to the possibility that certain nutrients might have leached out of the frozen–thawed *Artemia* before the larvae consumed them. Although the larvae consumed the frozen *Artemia*, they either showed lower assimilation efficiency or ingested less than larvae that were fed live *Artemia*.

Compared to other species, such as *Umbra krameri* (Esoxiformes) or cyprinids (Cypriniformes), the growth rate of *C. gobio* fed on *Artemia* is significantly slower, which is likely due to the species' biological characteristics and the lower rearing temperature (Table 4). However, it should be noted that the scientific literature reports significantly lower growth rates than ours, indicating that at temperatures between 14–16 °C, bullheads reach a length of 20 mm within 5–6 months and 40 mm within one year (Marieni and Anzani, 2018).

From a larval rearing technology perspective, we recommend feeding the Mixed group because frozen *Artemia* only needs to be stored and does not require daily initiation of live cultures. This significantly reduces labor requirements, which can be considerable in large-scale larval rearing operations.

In addition to the parent species *Cottus gobio*, twelve other *Cottus* species have been described (with EU 27 Red List Categories) that receive the same level of protection as the parent species. Although *Cottus gobio* is listed as Least Concern (LC) on the IUCN Red List, among the other species, some have been classified as Vulnerable (VU) or even Critically Endangered (CR), and three are listed as Data Deficient (DD). We believe that our findings can support the conservation efforts for all *Cottus* species, as there is significant overlap in their ecological characteristics and life history traits (Freyhof and Wright, 2011).

From 11 egg clutches originating from 23 adults (12 male, 11 female) — 6 used in the experiment and an additional 5 outside of it, a total of 751 feeding larvae were translocated to their parents' place of origin and to newly designated stream reaches as part of a conservation sampling program. Following the experiment, we delayed the release by ten days due to heavy rainfall in the target area, which would have created unfavorable conditions

Table 4. Summarized data about the experimental groups

Species		Feeding protocol	TL_0 (mm)	TLt (mm)	DGL (mm·day^{-1})	t (days)	T (°C)	Source
Scorpaeniformes	*Cottus gobio*	Fresh 2x feeding per day	9.1	14.5	0.26	21	13.5	Present study
		Mixed 2x feeding per day	9.1	14.7	0.27			
		Frozen 2x feeding per day	8.9	13.4	0.21			
Centrarchiformes	*Maccullochella peelii peelii*	Fresh 2x feeding per day	NA	14.3	NA	21	15.4	(Francis et al., 2019)
		Enriched 2x feeding per day	NA	16.1	NA			
Cypriniformes	*Barbus barbus*	Fresh 3x feeding per day	9.7	33.62	1.14	21	25	(Prusińska et al., 2020)
		Enriched 3x feeding per day	9.7	32.57	1.09			
Perciformes	*Dicentrarchus labrax*	DHAS	9.92	13.44	0.17	21	21.6	(El-Dahhar et al., 2024)
		FOVC	9.92	12.28	0.11			
		FOVE	9.92	14.22	0.20			
		FOCE	9.92	14.72	0.23			
		FO	9.92	11.94	0.10			
Siluriformes	*Corydoras aeneus*	Fresh 2x feeding per day	4.9	10.1	0.37	14	25.2	(Lipscomb et al., 2020)
	Synodontis eupterus		5.2	7.67	0.18			
	Synodontis nigriventris		3.63	5.85	0.16			
Cypriniformes	*Epalzeorhynchos bicolor*		3.37	6.71	0.24			
Perciformes	*Pterophyllum scalare*		4.65	6.23	0.11			
Anabantiformes	*Trichogaster lalius*		2.93	3.69	0.05			

Note: TL_0 = initial total length; TL_t = total length at time t; DGL = daily growth in length; t = days; T = temperature; DHAS = DHA selco® as a control group; FOVC = Fish oil +20% Vit. C; FOVE = Fish oil +20% Vit. E; FOCE = Fish oil + (10% Vit. C +10% Vit. E); FO = Fish oil.

for the released individuals. Ensuring their release under optimal conditions was crucial to minimize immediate negative impacts. Our estimated egg clutches contain, on average, 400 eggs, so there were ~ 4400 eggs and 751 feeding larvae introduced, which means 17.1% efficiency. The available literature sources have not tracked the relationship between the number of eggs per spawning event and the number of juveniles ultimately released. Therefore, no source indicating effectiveness was found, although all known breeding, release efforts and the survival success of the released individuals have been categorized as successful (Vught et al., 2010; Piccinini et al., 2012; Liefferinge et al., 2021). Consequently, we consider it important to monitor the released individuals, whose survival success would not only increase the population size but also expand the species' distribution range in the target area (Cheile Bicazului – Hășmaș National Park and Natura 2000 Site, Transylvania, Romania).

CONCLUSION

This study demonstrates that captive breeding and early larval rearing of *Cottus gobio* under controlled conditions is feasible and can yield viable offspring with relatively low mortality. The results highlight that diet quality significantly influences larval growth, with fresh or mixed *Artemia* diets supporting better development compared to frozen *Artemia* alone. A survival rate of 17.1% from egg to feeding larva stage provides a promising baseline for future conservation and restocking initiatives. These findings contribute valuable empirical data that can inform the development of effective propagation protocols for this species of conservation concern.

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