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Can We Turn Harmful Invasive Non-Native Fish Species into a Valuable Food Resource?

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Abstract: This study explores the potential of utilizing black bullhead (*Ameiurus melas* Rafinesque, 1820), an invasive freshwater species, as a stocking fish for aquaculture. Fish were mass-removed from Ponjavica Nature Park during two periods (2018–2019 and 2020–2021), with selected individuals reared to evaluate growth, survival, and meat quality. A total of 20,145 individuals were removed in the first period (168 reared), and 15,921 in the second (120 reared). Two rearing systems—cages and recirculating aquaculture systems (RAS)—and four feed types were tested. Results demonstrated the species' adaptability to intensive aquaculture, with good growth, resilience to high-protein diets, and tolerance to high stocking densities. Cage systems generally showed superior growth performance, while RAS produced higher survival rates. Both systems achieved favorable feed conversion ratios. Meat analysis revealed optimal levels of polyunsaturated fatty acids (PUFAs) in RAS and cage-reared fish, enhancing the species' nutritional value for human consumption. These findings demonstrate the feasibility of repurposing black bullhead as a sustainable aquaculture resource. This dual-purpose approach addresses ecological concerns while offering economic benefits through increased fish production and affordable, nutritious food availability. Further technological development is needed to optimize production systems for broader implementation.

Keywords: *Ameiurus melas*; freshwater ecosystems; aquaculture; quality of fish flesh

Key Contribution: This study highlights the nutritional benefits and market potential of black bullhead meat, while demonstrating how its sustainable farming can support native fish conservation and provide new economic opportunities in aquaculture.

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1. Introduction

Biodiversity, the basis of life on our planet, is being destroyed at a rate not recorded in history [1,2], and freshwater ecosystems are among the most endangered [3]. Despite well-documented threats, globally coordinated action to protect freshwater habitats is lacking, mainly due to the need for large-scale, multi-sectoral efforts [4,5].

Invasive species represent one of the significant threats to biodiversity globally [6–10], second only to habitat destruction [11–15]. Their introduction, often termed biological pollution [16], has more than doubled in recent decades, with non-native freshwater fish significantly impacting native biodiversity at multiple levels of biological organization [17]. Economic activities such as aquaculture (39%) and enhancement of wild stocks (17%) are the primary drivers of these introductions [18].

In Serbia, approximately 23% of fish species are non-native, mainly from North America, including the black bullhead (*Ameiurus melas* Rafinesque, 1820) [19]. This species dominates freshwater ecosystems in Serbia, negatively impacting native fish through predation on native fish species and competition for food with native fish species [12,20–22].

The EU regulates the management of invasive fish species under Regulation (EU) No. 1143/2014 [23] and the European Water Framework Directive (2000/60/EC) [24], with strategies varying by the member state. In Serbia, the Law on Protection and Sustainable Use of the Fish Fund [25] permits selective mass removal of non-native species, a practice included in Fisheries Management Programs. In reducing black bullhead's negative impact on the native ichthyofauna, its selective mass removal is considered an effective management measure and a potential solution to the problem [26–32]. In Serbia, several localities have organized recovery removals [30]. In 2023, the scientific results on the effects of selective mass removal on the native ichthyofauna and the population of black bullhead in Serbia were published for the first time, showing that its removal may have both positive and negative effects on species diversity, given that the abundance of particular native species has increased, but also the abundance of other invasive non-native fish species [32]. In previous mass removals, black bullhead individuals were not used for any purpose. These activities ended with digging holes in the shore into which fished-out individuals were placed, after which the holes were poured with lime. This inefficiency underscores the need for innovative solutions to manage invasive species while minimizing waste and maximizing benefits.

On the other hand, at the global level, catfish farming belongs to the top 10 species groups in global aquaculture during 2020, with an annual production of 6,019,881 t, and black bullhead is recognized as a viable aquaculture species due to its adaptability, low-fat, high-quality meat, and consumer appeal [33,34]. In the USA, cage, and pond culture are two forms of aquaculture in which black bullheads have been successfully bred [35]. In Europe, black bullhead is almost exclusively farmed in Italy, where it represents one of the most traditional systems of fish farming in the northern freshwater territories (along the Po River), where this fish species had a tradition as food supply and sport fishing [36,37]. To increase production and eliminate pathogens in catfish water, Italian farmers used more modern breeding systems, such as the recirculating aquaculture system (RAS), where the entire fattening cycle of the catfish was carried out under closed-cycle conditions until market size [36]. According to Bordignon et al. [38], the black bullhead may also be a compelling option for haloponics, especially in systems that require minimal technological input and environmental management.

This field-based study hypothesizes that removed black bullheads can be repurposed for aquaculture, turning an ecological problem into an economic and nutritional resource. Specifically, the research aimed to assess the yield efficiency of black bullheads in cage and recirculating aquaculture systems (RAS) using four types of extruded feed, addressing both ecological disruption and the low per capita fish consumption in Serbia (4.89 kg, compared to the global average of 20.2 kg) [39,40]. Increased consumption of this species may also contribute to reducing cardiovascular diseases in the human population, which are a leading cause of death in Serbia [41]. All these facts justified the assumption that removing black bullheads from natural ecosystems was worth cultivating in intensive systems [42]. Therefore, we aimed to assess yield efficiency using two different rearing systems (cages and RAS) and four types of extruded feed.

By establishing a system where removed black bullheads are raised to market size in fish farms and sold at competitive prices, the study seeks to create a self-sustaining model. Consequently, bullhead fisheries could finance continued mass removals of this species. In return, fish farm owners could get juvenile specimens. They would not have to invest in facilities and equipment for broodstock spawning and rearing, quickly getting high-quality fish of consumable size, which they could market at acceptable prices. This approach offers ecological benefits by reducing invasive populations, economic opportunities for fish farmers, and health benefits for consumers through affordable, nutritious fish access. The findings provide a foundation for integrating biodiversity conservation with sustainable food production, demonstrating the feasibility of transforming an invasive species into a valuable resource.

However, it is important to acknowledge that the economic valorization of invasive species such as *Ameiurus melas* raises critical ecological and ethical concerns. As Nuez et al. [43] highlighted, transforming an invasive species into a usable resource can undermine eradication efforts by creating conflicting incentives for its persistence or promoting its spread to new areas. These risks must be carefully considered and mitigated through strict regulatory control, including prohibitions on intentional breeding, limitations to individuals removed as part of authorized management actions, and traceability in distribution. Although our approach is based exclusively on the reuse of individuals removed from the natural environment within the existing Serbian legal framework, this broader perspective is essential to ensure that economic use does not compromise conservation objectives.

2. Materials and Methods

2.1. Sampling

The Ponjavica Nature Park is located in Vojvodina province of the Republic of Serbia (44°42′52″ N; 20°47′44″ E; altitude: 71 m). This locality was selected as the site for the selective mass removal of black bullhead. Ponjavica River is a slow-flowing plain river with a total length of 20 km, an average depth of 0.2 m, and a maximum depth of 2.5 m [44].

The selective mass removal of the black bullhead was carried out over four periods (August to October 2018, April to September 2019, August to October 2020, and April to September 2021) using cylindrical fyke nets with two conical-shaped funnel openings (length 85 cm, diameter 50 cm, 8 mm mesh). At each location, the fyke nets were positioned in three rows—on the banks' left and right sides of the banks and in the middle of the stream. Each row had five fyke nets connected by a rope at a distance of 5 m. On each sampling date, the fyke nets were set for 24 h and then checked daily for 4 days in 2018 and 3 days in 2019, 2020, and 2021.

All sampled fish were identified using the Handbook of European Freshwater Fishes [45] and Fishes of Serbia [46], and total length (TL) and total mass (TW) were measured at the nearest 0.1 cm and 1 g, respectively. During the selective removal, one part of the fished-out black bullhead specimens was transported for experimental rearing.

2.2. Experimental Rearing and Data Analysis

Two feeding experiments were conducted using individuals of different initial body weights, with the aim of reaching the consumable size for this species—defined as 250–300 g—and identifying the most suitable rearing technology for black bullheads.

Experimental rearing of black bullhead specimens was realized in the Center for Fisheries and Applied Hydrobiology (CEFAH) "Mali Dunav" (University of Belgrade—Faculty of Agriculture), on the Experimental Property "Radmilovac". The experiment was divided into July to October 2018 and June to October 2021. In the first period, the systems used were cages and RAS. The fish were fed with two types of extruded feed: Feed 1 (25/7

Standard), which contained 25% protein and 7% fat, and Feed 2 (32/7 Profi Effect), which included 32% protein and 7% fat. In the second period, the same systems (cages and RAS) were utilized, but the fish were given two types of commercial feed: Feed 3 (25/7), designed for carp, and Feed 4 (44/20), intended for trout. The amount of feed eaten was measured with an accuracy of 0.01 g. The detailed composition of all four diets is provided in the Supplementary Materials.

During both experiments, abiotic parameters were measured: temperature (T) in °C, oxygen (O_2) mg/L, pH, electrical conductivity (EC) in µS/cm, and dissolved oxygen concentration (DO) %, every day at 10 a.m. We measured these parameters using the MULTI 340i/SET instrument (WTW, Velbert, Germany).

During the first experiment, to uniform the conditions for the realization of the experiment, cages of the exact dimensions were placed in the RAS tanks and the pond. The cages used in both systems had identical dimensions of 0.6 m × 0.6 m × 0.6 m, corresponding to a total volume of 216 L. Each treatment (cages and RAS/Feed 1 and Feed 2) was conducted in triplicate. The experiment used six cages for the cage cultivation system (K1 to K6) and six for RAS (RAS 1 to RAS 6). Each of the cages was stocked with black bullheads so that each fish had a volume of 15 L of water at its disposal (density 1.23 kg/m^3). Thus, cages for the cage system and the RAS were stocked with 14 individuals, each with an average body weight of 19.11 ± 0.36 g (18.57–19.64 g). The pond used for cage culture had a surface area of 30 × 20 m (600 m^2), with an average depth of 1.4 m, resulting in a total volume of approximately 840 m^3. The experimental RAS consisted of individual tanks with a total volume of 1000 L. Each RAS tank was equipped with a mechanical filter, a biological filter, an oxygenation system, a UV disinfection unit, and a water heating system, allowing for precise control of environmental conditions. The experiment was performed for 90 days (16 July–16 October 2018). The fish in the experiment were fed twice a day (at 9 a.m. and 2 p.m.) with a daily meal of 2.2% relative to ichthyomass during the first 60 days. In the last 30 days, the amount of feed was reduced by 30% compared to the previous period due to falling water temperature and slower fish metabolism. Feed 1 was used for fish from odd numbers of the marked cages (K1, K3, and K5), cages in RAS (RAS 1, RAS 3, and RAS 5), and feed 2 was used for fish from evenly marked systems for cultivation (K2, K4, K6, RAS 2, RAS 4, RAS 6).

Throughout the second experiment, each of the three systems was stocked with black bullheads so that each fish had a volume of 21.6 L of water at its disposal (density 2.26 kg/m^3). Thus, cages for the cage system and the RAS were stocked with ten fish each, with an average body weight of 48.94 ± 0.38 g (48.26–49.88 g). The experiment was realized in 112 feeding days (25 June–17 October 2021). The fish in the experiment were fed twice a day (at 9 a.m. and 2 p.m.) with a daily meal of 2.5% relative to ichthyomass, except for the first 4 days, when the amount of feed was increased every day by 0.5% to get used to feeding and the last 23 days when they were fed with 2% due to falling water temperatures and slower metabolism of farmed fish. Carp feed, 25/7 (feed 3) was used for the steam-marked farming systems (K2, K4, K6, RAS 2, RAS 4, RAS 6, and trout feed 44/20 (feed 4) was used for the fish from oddly marked cages (K1, K3, and K5) and cages in RAS (RAS 1, RAS 3, and RAS 5).

Total length (TL), standard length (SL), total weight (TW), height (H), width (W), gutted weight (GW), and weight of liver and gonads were measured for all reared black bullhead individuals to the nearest 0.1 cm and 0.01 g. To assess the efficiency of black bullhead growth, the following indicators were monitored: fish growth (BWG) specific growth rate (SGR), average feed consumption per fish (FI), daily feed participation (DFR), feed conversion factor (FCR), metabolic growth rate (MGR) and survival rate (SR). These indices were calculated according to the formulas of Shamna et al. [47]. Fulton's coefficient

(K_f), Clark's coefficient (K_c), High–backedness index (I_h), Wide–backedness index (I_w), Gonadosomatic index (GSI), Hepatosomatic index (HSI), and Viscerosomatic index (VSI) were calculated according to formulas of Mohammad et al. [48] and Gabriel et al. [49].

Although no disease symptoms or unusual mortality patterns were observed during rearing, it must be emphasized that using wild-caught specimens in RAS entails potential health risks. We recommend implementing comprehensive pathogen screening and biosecurity protocols for future applications and research.

2.3. Methodology for Chemical Analysis of Black Bullhead Meat

At the end of the second experiment in 2021, black bullhead individuals were sampled from both rearing systems (10 fish from cages and 10 from RAS) for chemical composition and fatty acid analysis. Each fish's total weight and length were measured, and dorsal and adipose muscle tissues were dissected and frozen. These samples were not separated by diet but pooled within each rearing system due to analytical constraints and to reflect the overall influence of the production system on fillet quality. The control group consisted of individuals sampled at the beginning of the experiment, immediately after capture from the Ponjavica River, prior to stocking. This comparison allowed us to assess the impact of cultivation, as opposed to natural conditions, on the nutritional quality of the fish.

Protein (Kjeldahl nitrogen) was analyzed using a semi-automatic distillation unit (Kjeltec Auto 1030 Analyzer) with a block-digestion apparatus (Digestion System 20, Tecator, Höganäs, Sweden) according to the manufacturer's instructions (Tecator Manual Rev. 2.2). Analysis of lipid [50], moisture [51], and ash [52] was realized according to standard ISO methods. The results were conducted in triplicate.

2.4. Methodology for Fatty Acid (FA) Analysis of Black Bullhead Meat

2.4.1. Extraction of Total Lipids

Total lipids were extracted from fish fillets using accelerated solvent extraction (ASE) to assess FA content (ASE 200, Dionex, Sunnyvale, CA, USA). The operating conditions were identical to those described by Spirić et al. [53]. At 50 °C, the solvent from the collected extracts was extracted using a stream of nitrogen (Dionex Solvent evaporator 500, Dionex Corporation, Sunnyvale, CA, USA) until the extracts were dry.

2.4.2. Fatty Acids

According to [54], the fatty acid methyl esters (FAMEs) were made by transesterifying the lipids extracted by ASE with 0.25 M TMSH. An internal standard of 0.05 mL of heneicosanoic acid methyl ester (C21:0, c = 10 mg mL^{-1}, Fluka, Switzerland) was added before transesterification for measurement purposes. A Shimadzu 2010 gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with a split/splitless injector, a highly polar capillary column HP-88 column (J&W, Agilent, Santa Clara, CA, USA, 100 m × 0.25 mm × 0.2 μm), a flame ionization detector, and a workstation was utilized to examine the FAMEs. The injector and detector were heated to 250 and 280 °C, respectively. A preset column oven temperature, starting at 125 °C and finishing at 230 °C, was used to separate the tested chemicals. Detailed information on the GC's operating conditions was previously reported by Trbović et al. [55]. The signal-to-noise (S/N) ratio in gas chromatography was used to estimate the limit of detection (LOD) and the limit of quantification (LOQ). Specifically, the LOD was determined to be 0.05% and LOQ was 0.17%. The results were conducted in duplicate.

2.5. Statistical Analysis

All values are expressed as mean (x) ± standard deviation (SD). Before statistical analyses, all data sets were checked for normality using the Shapiro–Wilk test. Since all data sets lacked normality of the distribution, the non-parametric Kruskal-Wallis H test was used to assess differences among groups, followed by the Mann–Whitney U test. Significance for all conducted tests was considered at a level of $p \leq 0.05$. An ANOVA test was used with Dunnett's method to compare the composition of fatty acids to determine mean values relative to the control. Statistical analyses were performed in the Statistica 7.0 Software (StatSoft, Inc., Tulsa, OK, USA).

3. Results

3.1. Abiotic Parameters

A total of 20,145 individuals were removed from Ponjavica River during the first mass removal in 2018 and 2019, of which 168 individuals were taken to CEFAH for experimental rearing. In both systems (cages and RAS), individuals had an average TW of 19.1 g (18.6–19.6 g). During the 2018 experiment, significant differences were found between cages and RAS for the following parameters: T, O_2, pH, and DO. The water in the RAS system was more basic and richer in oxygen, while its temperature was lower compared to the cage system. Within the RAS system, no statistically significant differences were recorded between replicates (cages) for the examined parameters (Table 1). To facilitate comparison, abiotic parameters from both experimental years are presented in a unified table format (Table 1). However, all statistical analyses were performed exclusively within each experimental year (2018 or 2021), comparing rearing systems (cages vs. RAS) without cross-year or diet-based comparisons.

Table 1. Results of abiotic water parameters during experimental rearing in cages and RAS in 2018 and 2021. Values are presented as mean ± SD (minimum and maximum values are given in parentheses).

Year	2018		2021	
Abiotic Parameters	**Cages Mean ± SD (Min–Max)**	**RAS Mean ± SD (Min–Max)**	**Cages Mean ± SD (Min–Max)**	**RAS Mean ± SD (Min–Max)**
Temperature (°C)	22.2 ± 4.65 [a] (14.3–28.2)	19.03 ± 2.18 [b] (14.1–24)	20.69 ± 4.79 [a] (10.3–28.4)	18.82 ± 2.8 [b] (11.6–24.9)
Oxygen (mg/L)	2.82 ± 0.88 [b] (1.51–4.36)	5.46 ± 0.84 [a] (4.46–8.45)	8.04 ± 1.94 [b] (3.52–11.8)	9.09 ± 0.98 [a] (6.17–12.22)
Oxygen saturation (%)	33.83 ± 10.78 [b] (15.4–55.4)	58.98 ± 11.62 [a] (45.6–94.2)	85.67 ± 23.71 [b] (41.5–139.3)	97.12 ± 10.09 [a] (62.7–126.7)
pH	8.25 ± 0.21 [b] (7.94–8.74)	8.75 ± 0.15 [a] (8.28–8.95)	8.72 ± 0.52 (7.69–9.6)	8.8 ± 0.39 (7.87–9.51)
Electrical conductivity (µs/cm)	2052.78 ± 247.16 [a] (1150–2250)	2106.72 ± 55.07 [a] (1912–2270)	2430 ± 0.17 [a] (2020–2990)	2280.4 ± 97.34 [b] (2040–2590)

[a, b] Different letters (a, b) within a row indicate statistically significant differences between cage and RAS systems within the same year (Mann–Whitney U test, $p \leq 0.05$).

A total of 15,921 individuals were removed during the second mass removal from the same locality in 2020 and 2021, of which 120 were taken to CEFAH for experimental rearing. In both systems (cages and RAS), individuals had an average TW of 48.9 g (48.7–49.1 g). During the 2021 experiment, statistically significant differences were found between cages and RAS systems for the following parameters: T, O_2, EC, and DO. The water in the RAS system was richer in oxygen, while its T and EC were lower compared to the cage system (Table 1).

3.2. Growth Performance and Survival of Black Bullhead

The results of the experimental rearing in 2018 showed that there was no difference in initial body mass between the cages and RAS. At the end of the experiment, there were significant differences between the initial and final body weight in both systems. Significant differences were found in the final mass of individuals between the cages and RAS. At the end of the experiment in the cage-rearing system, the individuals fed by feed 1 had no significant mass differences compared to those that consumed feed 2. The same was noted for individuals reared in RAS (Table 2).

Table 2. Growth performance and survival of black bullhead. Fish growth (BWG), specific growth rate (SGR), average feed consumption per fish (FI), daily feed participation (DFR), feed conversion rate (FCR), metabolic growth rate (MGR), and survival rate (SR). Values are presented as mean ± standard deviation (SD).

Year	**2018**				**2021**			
Rearing System	**Cage**		**RAS**		**Cage**		**RAS**	
Treatment	**Feed 1**	**Feed 2**	**Feed 1**	**Feed 2**	**Feed 3**	**Feed 4**	**Feed 3**	**Feed 4**
Initial body mass (g)	18.9 ± 0.0	19.3 ± 0.0	18.9 ± 0.4	19.3 ± 0.6	48.7 ± 0.5	49 ± 0.8	49 ± 0.1	49.1 ± 0.1
Initial body length (cm)	/	/	/	/	16.6 ± 0.2	16.5 ± 0.3	17.3 ± 0.1	17.3 ± 0.4
Final body mass (g)	60.3 ± 9.3	69.3 ± 11.7	41.9 ± 3.1	42.1 ± 2.8	226.2 ± 33.8	196.3 ± 8.7	200.5 ± 26.8	150.8 ± 11.4
Final body length (cm)	/	/	/	/	24.3 ± 0.9	23.7 ± 0.8	23.3 ± 1.2	21.2 ± 1.0
BWG (g)	41.4	50.1	23.0	22.8	177.5	147.3	151.5	100.8
SGR	1.3	1.4	0.9	0.8	1.4	1.2	1.2	1.0
>FI (g d^{-1})	1.9	0.9	0.5	0.5	4.7	4.1	3.4	4.6
DFR (%)	2.6	1.8	1.7	1.7	2.0	2.0	2.4	2.6
FCR	1.4	1.5	2.1	2.0	2.4	2.4	2.0	2.4
MGR_{MBW} (g $kg^{-0.8}$ d^{-1})	14.0	11.1	7.0	7.0	7.2	7.1	11.9	9.2
SR	38.1	73.8	97.6	100	20.0	23.3	63.3	25.00

The results of the 2021 experimental rearing showed no significant difference in initial body mass between fish in cage systems and those in RAS. However, significant differences were observed in mass, length, and condition of fish between the two systems when fed with feed 3 and feed 4. For individuals fed with feed 3, no significant differences in mass were noted at the start between the cage system and RAS. Similar observations were made for individuals fed with feed 4. No significant differences in weight were observed between fish fed with feed 3 and those fed with feed 4 within the cage system (Table 2).

After three months of black bullhead rearing in 2018, significantly better growth was recorded in the cage system compared to the RAS, but the survival rate in the RAS was, on average, 1.8 times higher than in the cage system. During the first experiment, the mortality rate of black bullheads in the cage system was 44%. Out of 84 total black bullhead individuals (14 in each cage), 11 died during the first month, 26 during the second, and no individual died during the third month of rearing. Much better results were obtained in RAS. In this system, the mortality rate of the black bullhead was only 1.2%. Out of 84 black bullheads (14 in each cage), only one individual died during the second month of rearing.

Although the results from 2021 showed that, in general, the individuals from the cage system had better conditions than those from the RAS, the average mortality in the cage system was two times higher compared to the RAS. During the second experiment, the mortality rate of black bullheads in the cage system was 78%. Out of 60 total black bullheads (10 in each cage), 43 died during the first month, three during the second, and one individual died during the third month of breeding. Once again, much better results were obtained in RAS. In this system, the mortality rate of the black bullhead was 45%. Out of 60 total black bullhead individuals (10 in each cage), 8 died during the first month, 14 during the second, 4 during the third month, and 1 during the fourth month of breeding (Table 2).

At the end of the experiment, no significant differences in mass between the cage system and RAS were found in the individuals fed with feed 4. No differences in length were found either, but individuals from the cage system were in better condition than the RAS. During the final measurement of individuals reared in RAS, individuals fed with feed 3 had significantly lower masses, lengths, and fitness than those fed with feed 4 (Table 2).

Experimental rearing in 2018 showed that regardless of the type of feed, significantly higher values of BWG, SGR, FI, DFR, and MGR were recorded in the cage system compared to RAS, while the opposite was recorded for FCR and SR (Table 2).

During the experimental rearing in 2021, significantly higher values of BWG compared to RAS were recorded in the cage system regardless of the type of feed, while the opposite was recorded for SGR, DFR, MGR, and SR. Irrespective of the rearing system (cage system or RAS), no significant differences were found for the investigated coefficients concerning the type of feed used during the experiment (Table 2).

Regardless of the type of feed, significantly higher values of the K_f, K_c, I_h, and I_w were recorded in the cage system compared to RAS. Individuals from the cage system fed with feed 4 had significantly higher values of K_f, K_c, I_h, and I_w, as well as HSI, VSI, and GSI, compared to individuals from RAS who consumed the same type of feed. Individuals from the cage system fed with feed 3 had significantly higher values of K_c, I_h, and I_w compared to individuals from RAS who consumed the same type of feed. Regardless of the experiment (cage system or RAS), individuals who consumed feed 4 had significantly higher K_f and K_c values and HSI values than those who consumed feed 3. As for the cage system, individuals who consumed feed 4 had substantially higher K_f, K_c, I_w, and VSI values and lower Ih values. As for the RAS, individuals who consumed feed 4 had significantly higher K_f, K_c, and I_w values and lower HSI, VSI, and GSI (Table 3).

Table 3. Results of Fulton's coefficient (K_f), Clark's coefficient (K_c), High–backedness index (I_h), Wide–backedness index (I_w), gonadosomatic index (GSI), hepatosomatic index (HSI), and viscerosomatic index (VSI) during experimental rearing in 2021. Values are presented as mean ± SD.

	Feed 3		Feed 4	
Indices	**Cage 1**	**RAS 1**	**Cage 2**	**RAS 2**
K_f	1.4 ± 0.1 ^{a}	1.3 ± 0.1 ^{b}	1.8 ± 0.2 ^{A}	1.4 ± 0.1 ^{B}
K_c	1.2 ± 0.1	1.1 ± 0.1	1.5 ± 0.2 ^{A}	1.3 ± 0.1 ^{B}
I_h	5.2 ± 0.4 ^{a}	3.9 ± 0.2 ^{b}	4.4 ± 0.4 ^{A}	3.9 ± 0.4 ^{B}
I_w	18.5 ± 1.4 ^{a}	14.9 ± 1.4 ^{b}	20.3 ± 1.2 ^{A}	16.3 ± 1.4 ^{B}
GSI	0.3 ± 0.3	0.9 ± 0.7	0.7 ± 0.5 ^{A}	0.2 ± 0.1 ^{B}
HSI	3.6 ± 0.4	4.1 ± 0.4	4.0 ± 0.4 ^{A}	3.1 ± 0.3 ^{B}
VSI	9.9 ± 1.2	11.1 ± 1.3	12.0 ± 1.7 ^{A}	9.7 ± 1.1 ^{B}

$^{a, b}$ Values with different letters in the same row indicate statistically significant differences (Mann–Whitney U_{TecT}, $p \leq 0.05$) between Cage and RAS where feed 3 was applied. $^{A, B}$ Values with different letters in the same row indicate statistically significant differences (Mann–Whitney U_{TecT}, $p \leq 0.05$) between Cage and RAS where feed 4 was applied.

3.3. Chemical Composition of Black Bullhead Meat

The chemical composition of the black bullhead is presented in Figure 1. Significantly higher protein (Figure 1a) and lipid (Figure 1b) content in the meat of black bullhead was in RAS and cage system compared to control. The moisture content in fish meat was significantly lower in the RAS and cage system than in the control (Figure 1c). The ash contents differed considerably in the meat of black bullheads in RAS and cage systems compared to those in control (Figure 1d).

Monounsaturated fatty acids (MUFAs) represented the predominant group, followed by polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs). In this study, SFAs were significantly lower in black bullhead meat in the RAS and cage system than in the control. The most common SFAs were palmitic acid (C16:0). The MUFAs were significantly higher in black bullhead meat in RAS and cage systems compared to the control. The most common was oleic acid (C18:1n-9). The PUFAs were considerably higher in the RAS and cage system than in the control. From the n-6 PUFA, the most common was linoleic acid (C18:2n-6), and from the n-3 PUFA, the most common was linolenic acid (C18:3n-3) (Table 4).

Table 4. Results of black bullhead meat's fatty acid composition (g 100^{-1} g) at the end of the second experimental rearing in 2021.

Fatty Acid	Control	Cage 3	Cage 4	RAS 3	RAS 4	*p*
C14:0	1.14 ± 0.01	0.53 ± 0.01	0.45 ± 0.01	0.43 ± 0.01	0.37 ± 0.01	***
C15:0	0.42 ± 0.01	0.24 ± 0.02	0.14 ± 0.02	0.06 ± 0.01	0.07 ± 0.01	***
C16:0	24.30 ± 0.01	14.39 ± 0.01	13.55 ± 0.02	15.11 ± 0.03	14.88 ± 0.03	***
C16:1	3.19 ± 0.01	1.99 ± 0.02	1.88 ± 0.01	1.97 ± 0.03	1.78 ± 0.04	***
C17:0	0.50 ± 0.01	0.26 ± 0.01	0.17 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	***
C18:0	7.89 ± 0.02	3.54 ± 0.01	3.82 ± 0.01	3.81 ± 0.01	3.00 ± 0.01	***
C18:1n-9	26.25 ± 0.03	35.50 ± 0.05	36.92 ± 0.03	37.90 ± 0.05	38.74 ± 0.01	***
C18:2n-6	21.02 ± 0.18	30.32 ± 0.01	28.92 ± 0.02	31.96 ± 0.03	32.68 ± 0.02	***
C20:0	0.44 ± 0.01	0.30 ± 0.01	0.30 ± 0.01	0.28 ± 0.04	0.20 ± 0.04	**
C18:3n-6	0.37 ± 0.02	0.44 ± 0.03	0.39 ± 0.01	0.73 ± 0.02	0.78 ± 0.01	NS
C18:3n-3	2.18 ± 0.01	6.49 ± 0.02	7.86 ± 0.01	3.34 ± 0.01	4.21 ± 0.01	***
C20:1	1.26 ± 0.01	1.34 ± 0.01	0.18 ± 0.01	1.04 ± 0.02	0.09 ± 0.01	**
C20:2n-6	0.99 ± 0.01	1.25 ± 0.02	0.98 ± 0.01	0.92 ± 0.03	0.72 ± 0.04	NS
C20:3n-6	1.21 ± 0.01	0.75 ± 0.02	0.70 ± 0.02	1.04 ± 0.03	0.88 ± 0.03	NS
C20:3n-3	0.26 ± 0.01	0.35 ± 0.02	0.60 ± 0.02	0.12 ± 0.03	0.15 ± 0.02	*
C20:4 n-6	2.78 ± 0.01	0.66 ± 0.03	0.40 ± 0.04	0.61 ± 0.03	0.52 ± 0.02	***
C20:5n-3	1.53 ± 0.01	0.69 ± 0.01	0.85 ± 0.02	0.12 ± 0.04	0.21 ± 0.02	***
C22:5n-3	1.18 ± 0.01	0.49 ± 0.19	0.39 ± 0.07	0.20 ± 0.08	0.35 ± 0.52	NS
C22:6n-3	3.07 ± 0.11	0.48 ± 0.03	0.40 ± 0.01	0.23 ± 0.01	0.25 ± 0.01	***
SFA	34.69 ± 0.10	19.26 ± 0.10	18.43 ± 0.10	19.81 ± 0.14	18.64 ± 0.12	***
MUFA	30.70 ± 0.13	38.83 ± 0.14	38.20 ± 0.14	40.91 ± 0.14	38.83 ± 0.14	**
PUFA	31.83 ± 0.24	41.25 ± 0.29	41.49 ± 0.40	38.67 ± 0.49	40.75 ± 0.64	**
n-6	23.60 ± 0.23	32.76 ± 0.38	30.30 ± 0.24	34.65 ± 0.40	33.92 ± 0.58	**
n-3	8.22 ± 0.09	8.49 ± 0.14	10.10 ± 0.12	4.02 ± 0.14	5.17 ± 0.16	**
n-6/n-3	2.87 ± 0.25	3.86 ± 0.12	3.00 ± 0.19	8.62 ± 0.49	6.56 ± 0.33	*

Data are presented as mean ± SD; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS—not significant differences $p > 0.05$.

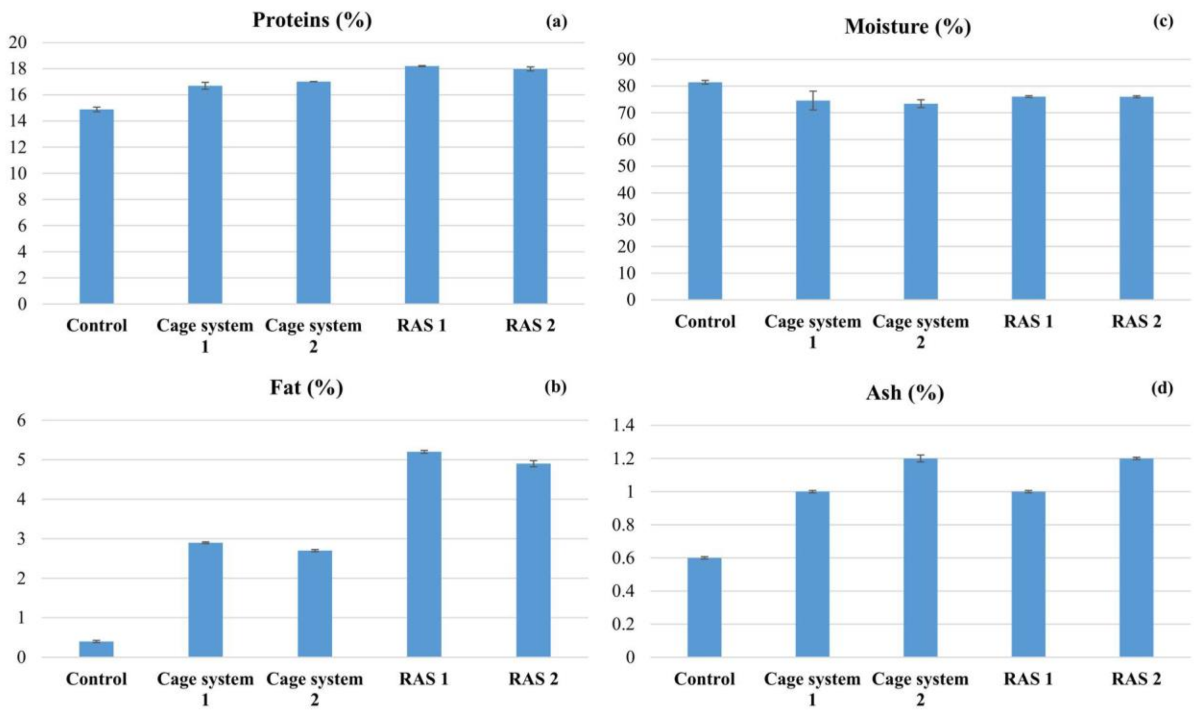

Figure 1. Results of the chemical composition of black bullhead meat at the end of the second experimental rearing in 2021. (**a**) Crude protein content (%); (**b**) Total lipid content (%); (**c**) Moisture content (%) and (**d**) Ash content (%). Each bar represents the mean ± SD.

4. Discussion

Water temperature, dissolved oxygen concentration (DO), and photoperiod affect fish growth and metabolism, as reported by Buentello et al. [56]. Therefore, it is essential to monitor and maintain them at an optimal level for the organism's feed intake and growth rates to be both high and uniform [56]. During both experiments, water temperature showed more significant fluctuation in cages than RAS. These results were similar to the ones during experimental rearing in the pond (24.11 ± 4.19 °C) and in RAS (20.48 ± 0.9 °C) in Italy [36] as well as in cages (21.7 ± 0.3 °C) and ponds (21.8 ± 0.3 °C) in the USA [35]. Cold-blooded animals depend heavily on the ambient temperature, and conditions below or beyond the thermal limit might cause changes to the fish's immune system [57–59]. According to Baby et al. [58], the ideal temperature range for channel catfish (*Ictalurus punctatus*) growth was higher and ranged between 26 and 32 °C. A study on the temperature requirements for raising channel catfish from fingerlings to market size showed that the best food conversion ratio and growth were observed between 28 and 30 °C [58]. A study from the USA confirmed that the best food conversion for black bullhead was obtained when the water temperatures were between 23 °C and 24 °C [60]. Also, a study evaluating the effects of water salinity in an aquaponic system with rainbow trout (*Oncorhynchus mykiss*), black bullhead, Swiss chard (*Beta vulgaris*), and cherry tomato (*Solanum lycopersicum*) showed that black bullhead showed poor growth, regardless of water salinity, when the water temperature was less than 17 °C [38].

During the 2018 experiment, mean DO values were significantly lower compared to 2021 in cages and RAS. The 2021 values were much closer to those recorded during experimental rearing in Italy [36]. The results from RAS in 2018 were close to the results of experimental breeding in the USA [35]. The pH values (Table 1) were slightly higher than those during experimental rearing in Italy [36,38]. However, these values are very similar to the laboratory values (pH 8.24) during the experimental breeding of brown bullhead from the fingerlings in the RAS in Debrecen [34] and also to the pH results from the USA, which averaged 8.2 ± 0.2 for both cages and ponds [35].

Unlike the experiment conducted in CEFAH, during experimental rearing in Italy, no significant differences were noted between the final mean body weight of specimens reared in ponds and those that were reared in indoor fiberglass tanks, each associated with a recirculating filtration system. Fish from the cage system adopted both types of feed better than fish from the RAS, as seen in earlier studies, though similar patterns were not observed during experiments in Italy. The growth of fish during this experiment was higher compared to our research, 137.6 g in ponds (PN) and 146.4 g in indoor tanks working in closed recirculated systems (RC) [36]. Fish grew consistently with both types of feed in our research, which was expected, aligning with the Italian and American findings. Regarding these results, the experiment performed in Italy lasted twice as long as the experiment in 2018 and 69 days longer than the experiment in 2021. In another experiment in North America, which lasted 117 days, it was concluded that this species reared in open ponds showed better growth and feed conversion than fish reared in cages. Black bullhead individuals gained between 59.1 and 82.7 g in the cages and 134.3 and 176.5 g in the ponds [35]. At the beginning of our experiment, significant differences in length and condition were found between fish in the cage system and those in RAS, with RAS fish being longer. However, these fish had lower conditions than those in the cage system, highlighting the trade-offs in rearing environments. The results for 2018 are significantly lower compared to these results, but the results for 2021 are much closer. Another experimental rearing of this species was described more than 50 years ago in small ponds in eastern South Dakota. Rearing lasted three months, and during that time, the fish gained approximately 82 g (120 to 232 g) in the large cages, while the fish in the small cages gained 100 g (128 to 228 g). Over 75 percent of the total weight gain occurred in the first six weeks [60]. By the end of the experiment in 2021, statistically significant differences in mass were found for fish fed with feed 3, with those in the cage system having greater mass, a trend consistent with prior studies. During the study that evaluated the role of increasing salinity in brackish-water aquaponics on the growth of rainbow trout, black bullhead, Swiss chard, and cherry tomato, overall weight gain was meager, considering that the experiment lasted 268 days and amounted to 41 g (from 147 ± 22 g to 192 ± 50 g) [38].

It is important to emphasize that the stocking densities applied in this study (1.23–2.26 kg/m^3) were considerably lower than those commonly used in commercial aquaculture systems, which typically range between 10 and 50 kg/m^3 and can exceed 200 kg/m^3 in highly intensive farming (e.g., African catfish). These lower densities were intentionally chosen due to the experimental nature of the study and the fact that all reared individuals were wild-caught, with unknown prior health status. We aimed to assess the species' basic adaptability, growth performance, and survival under controlled, low-stress conditions, minimizing potential adverse effects of crowding and disease. While this approach provided important baseline data, it does limit the direct applicability of the results to high-intensity commercial production. Therefore, we recommend that future research explore rearing performance under higher stocking densities, enabling a more accurate evaluation of yield efficiency and economic feasibility in real-world aquaculture contexts. Experimental rearing in Italy showed results of the SGR 1.8 ± 0.5 in PN (refers to fish reared in three 1000 m^2 ponds) and 1.9 ± 0.3 in RC (individuals reared in three 2 m^3 indoor tanks operating under a closed recirculating (RAS) system) [36]. Notably, fish fed with feed 4 from both systems exhibited better conditions in our research, emphasizing its potential effectiveness across different systems. Our results were lower compared to those from Italy. Also, SR results during experimental rearing in Italy were significantly higher compared to our results (from 20% to 73.21% in ponds and from 25% to 100% in

RAS) and amounted to 86.6% (PN) and 99% (RC) [36]. These values were also significantly higher during the experiment in the USA and averaged 93.9% (from 91.8 to 96.2%) in cages and 91.3% (from 88.5 to 92.7%) in ponds [35]. However, the FCR results of rearing carried out in CEFAH were significantly higher than those during rearing in Italy, which were 1.2 for PN and 1.1 for RAS [36]. The feed conversion index during black bullhead rearing in Italy generally ranges between 1.2:1 and 1.5:1 [61]. According to Robinson and Li [62], smaller channel catfish developed quicker, converted feed more effectively, and consumed more feed as a percentage of body weight than larger fish. This is consistent with the fundamental principle of animal nutrition, which says that young, smaller animals usually develop quicker and consume feed more efficiently than older, larger animals [62]. Our research showed the opposite results—in cages, smaller individuals (in 2018) had lower FCR values than larger individuals (in 2021), while these values were pretty equal in RAS during both rearing years.

The high variability in survival rates between rearing systems and experimental years can be attributed to several interrelated factors. In the second experiment, fish were significantly larger at stocking and exposed to nearly double the biomass density compared to the first experiment. This likely increased physiological stress and competition for limited resources, particularly in cages, where environmental conditions are less controlled than in RAS. Furthermore, older fish are generally less adaptable to abrupt changes in habitat conditions, making their transition from natural environments to intensive rearing systems more challenging. Although advantageous in terms of growth, cage systems remain more vulnerable to external environmental fluctuations (e.g., temperature shifts, dissolved oxygen drops), predation, and physical disturbances, all of which may contribute to elevated mortality.

In contrast, RAS provides more excellent environmental stability and biosecurity, lowering mortality rates. The unexpectedly high mortality in cages, particularly in the second experiment (78%), suggests that technical improvements such as lower stocking densities, longer acclimation phases, improved cage design, and frequent environmental monitoring are necessary. Despite these limitations, the study demonstrates that successful cultivation of black bullheads is achievable, particularly under optimized RAS conditions. These results do not negate the feasibility of using mass-removed individuals for aquaculture but highlight the need for refined protocols. The high growth performance and feed efficiency observed justify continued exploration, with a focus on technological innovation to minimize mortality and enhance the viability of cage-based rearing.

Values of Fulton's coefficient during black bullhead rearing in Italy were almost identical, and at the end of the experiment, they were 1.34 in PN and 1.35 in RC [36]. They were close to the average value in RAS and lower than those in cages. The natural populations of this species were studied in the Ponjavica Nature Park (in 2018 and 2019). In Lake Sava (from 2009 to 2012), this coefficient's values were close to those during rearing in CEFAH, averaging 1.13 ± 0.24 and 1.33 ± 0.06, respectively [21,32]. For the black bullhead, the highest condition index was observed in fish farmed at medium salinity (1.61), while the lowest was in those kept with high salinity (1.52). These findings suggest that the black bullhead catfish probably does best in low-salinity waters (0.5‰ to 3‰), similar to other stenohaline species like the channel catfish [38]. The average GSI values were almost identical in cages and RAS. They were significantly lower than in the natural populations in the Ponjavica Nature Park and Lake Sava, averaging 1.02 ± 0.5 and 1.33 ± 0.52, respectively [21,32]. The average GSI value across other European non-native populations is 2.14 [63]. The HSI values were quite different, i.e., they were significantly higher during breeding in CEFAH than in the natural population in the Ponjavica Nature Park and at Lake Sava, where they averaged 2 ± 0.16 and 2.74 ± 0.47, respectively [21,32].

Concerning lipid content, the meat of bullhead from RAS and cage system could be considered moderately fatty fish meat (with a fat content of 4–8%) [64]. The results of proximate composition in cages obtained from black bullheads are very similar [38]. Fat content values were 1.7–2.1, lower than what we published for the cage system. However, the moisture content (77.5–76.6) was higher than ours in the cage system (73.37–74.59). Several studies have demonstrated the health benefits of PUFAs to humans [65,66]. Docosahexaenoic acid (C22:6n-3, DHA) and eicosapentaenoic acid (C22:5n-3, EPA) were significantly higher in control and the RAS and cage system. The protein, fat, and fatty acid profiles in the cage and RAS systems were superior to those observed in the pond system (control). Both the cage and RAS systems had higher levels of proteins and fats. Furthermore, these systems contained lower amounts of SFA and higher amounts of MUFA and PUFA, particularly linolenic acid (C18:3n-3). The experiment showed that individuals fed with feed 4 had more n-3 than those fed with feed 3. These fatty acids have many physiological properties. They affect the immune system, regulate blood flow, support immune response, and reduce the risk of coronary disease [67,68]. The n-6/n-3 ratio was in the cage system between 3 and 3.86 and in RAS between 6.56 and 8.62. An optimal n-6/n-3 ratio, which is 4:1, is desirable to reduce the risk of many diseases in human consumers [69], and the average value in cages is below this value, while in RAS, it is above that value. The results of fatty acid composition in cages obtained from black bullhead are very similar to those presented in the paper of Bordignon et al. [38], although to some extent in a different experiment. Published values of n-6/n-3 ratio were 2.27–2.32. This value is lower than what we have published for the cage culture system (3.00–3.86).

Although some wild-caught black bullheads may reach marketable size, their immediate use poses several concerns, including variability in flesh quality, uncertain consumer acceptance, and potential health risks. Introducing wild individuals into closed aquaculture systems, such as RAS, carries the inherent danger of pathogen transmission. While no signs of disease or elevated mortality were observed during our experiments, the absence of clinical symptoms does not exclude the possibility of latent infections. Previous studies have reported severe outbreaks in Ameiurus species caused by pathogens such as ranavirus and herpesvirus, resulting in high mortality across European catfish farms [70–74]. Controlled rearing offers the advantage of biosecurity oversight, health screening, and improvement of product uniformity, which together enhance food safety and market value. For these reasons, we strongly recommend the implementation of strict biosecurity protocols and continuous health monitoring when cultivating wild-caught stocks in intensive systems.

5. Conclusions

The initial hypothesis of the research was to explore whether the invasive black bullhead can be used as stocking fish, which solves the ecological problem caused by the overpopulation of this non-native species, and then to use this species as a valuable resource in human nutrition. The findings from the study provided several intriguing conclusions:

1. Adaptability and Growth: Both experimental setups (cage systems and RAS) demonstrated that the black bullhead adapts well to intensive fish production environments. It successfully grows in these systems, thriving on high-protein food and tolerating high population densities and stress. Notably, the cage system was generally more effective for rearing black bullheads, although the survival rate in RAS was over twice as high compared to cages. The black bullhead exhibited good growth parameters and feed conversion rates in both systems.
2. Nutritional Composition: The meat of black bullhead was found to have an optimal ratio of polyunsaturated fatty acids (PUFAs), particularly the n-6/n-3 ratio, which is

beneficial for cardiovascular health. PUFAs were significantly higher in RAS and cage systems than in control.

3. Public Health: The favorable omega-6 to omega-3 fatty acid ratio in black bullhead meat suggests that increased consumption could help reduce cardiovascular diseases.
4. Efficiency and Potential: The research achieved its goals, demonstrating yield efficiency in cage and RAS systems with different types of extruded feed. It also highlighted potential challenges in breeding black bullheads and the need to improve technological conditions in future rearing systems. If production proves profitable, it could enhance fish production in Serbia and increase consumer access to affordable fish. Using juveniles from native populations could help keep market prices reasonable.
5. Ecosystem Impact: The research underscores how a non-native, invasive species can be transformed into a valuable food source, aiding in ecosystem management by removing the species from native habitats where it causes ecological disruption.
6. Economic Viability: Developing a fishing, farming, and marketing black bullhead system could be economically viable. It offers a self-sustaining model that benefits fish farmers and consumers, potentially minimizing the need for additional investments in breeding facilities. However, to avoid unintended ecological consequences, any economic valorization of invasive species such as black bullhead must be accompanied by stringent regulatory measures aimed at preventing their further spread, including restrictions on intentional breeding and mandatory traceability protocols for harvested individuals.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes10050207/s1, Table S1: Formulation and approximate composition of diets.

Author Contributions: M.J. conceived the study's idea and led both projects. M.S. and Z.M. designed the methodology for experimental rearing and performed the laboratory work. M.J., M.S.-L. and D.N. collected samples and contributed to the organization's logistics and realization of all fieldwork activities. D.N. and D.T. performed statistical analyses. D.T. performed laboratory work concerning chemical and fatty acid analysis of black bullhead meat. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The authors received authorization from the Ministry of Environmental Protection and the Environmental Protection Agency of the Republic of Serbia to conduct the research. During the sampling and research, animal welfare was strictly taken into account. A special permit from the ethical commission was not required, given that these invasive fish species must be removed from the environment.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare that they have no conflicts of interest.

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