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Ecological Impact of Invasive Fish Species on Species and Ichthyocenotic Associations in Freshwater Aquatic Ecosystems of Romania †

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Abstract: The authors provide a list of the alien fish species that have entered the Romanian ichthyofauna fauna and specify the year, place of signaling and the ecological impact of the respective species. Further on, the authors describe some behavioral aspects of the following species: *Lepomis gibbosus* (pumpkin seed sunfish), *Carassius auratus gibelio* (silver crucian carp) and *Pseudorasbora parva* (topmouth gudgeon).

Keywords: invasive species; ichthyocenotic associations; freshwater ecosystems; ritualized stage; aggressive stage

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1. Introduction

The issue of the effect of alien species when they are introduced outside their natural range in aquatic and terrestrial habitats on Earth has only become a scientific concern in recent decays, although many specialists have made efforts and realized the Convention on the Conservation of Migratory Species of Wild Animals (CMS) on 23 June 1979, in Bonn, Germany [1–3].

In 1999, at the University of Montpellier, France, several specialists from all over the world, namely those working groups already with a tradition in research on the impact of invasive alien species on native species since the 1970s from Germany, France, Australia and New Zealand met to discuss and even modify some articles of the CMS [1–3].

In January 2000, the International Biodiversity Conservation Academy on the Island of Vilm, Germany, in the Baltic Sea organized, together with the German Federal Agency for Nature Conservation and the SBSTTA, a seminar for amendments to the CMS and the realization of a pan-European strategy, or even a special EU Invasive Species Convention or annex, to be included in the EU Natura 2000 program and to be the legal act to dynamize and reorient all taxonomists towards further research to quantify as accurately as possible the impact of alien species on native species and the ecological balance of the ecosystems in which they have entered [1–3].

The Bern Convention initiative for a European Strategy on Invasive Alien Species, in collaboration with the European Section of the IUCN Invasive Species Specialist Group, began in 2000. It has been welcomed by the Second Intergovernmental Conference on Biodiversity in Europe in Budapest and the CBD [1–3].

The European Strategy on Invasive Alien Species boosts research in this field and allows special funding for this type of scientific research, at least for EU member countries and those that can access EU funds for basic and applied scientific research. The CBD has included many articles of the CMS, translated into specific articles and amendments on invasive alien species, which took place from 1999 to 2004 and through the organization of several seminars and meetings of naturalists within the European SBSSTA [1–3].

Concerning the introduction of alien species of fish in the ichthyofauna of Romania, it is considered that there are two main periods: the first period of acclimatization of new fish species dates from the earliest times and until 1956, the year when the first paper in Romanian language appeared about the acclimatization of valuable fish species in our waters and also the year when the first batch of embryonated eggs of Peipsi whitefish (*Coregonus lavaretus*) was brought for acclimatization; and the second period starts in 1956 and continues until today [4].

The fish species introduced into the ichthyofauna of Romania in freshwater habitats have been relatively little studied in our country [5,6]. Below we present a list of foreign species of fish that were introduced or natural penetrated into Romania [7–10] (Table 1).

Table 1. Foreign species of fishes introduced or natural penetrated into Romania.

Crt. No.	Species	Date and Place of Introduction	Purpose of Introduction	Effects Produced and Reported
1.	*Oncorhynchusmikiss*	1885, anonymous [9]	Aquaculture	In the trout farms
2.	*Salvelinusfontinalis*	1906, Moldova River [5,6,9]	Aquaculture	Stabilized
3.	*Carassius auratus gibellio*	1912, Moldova River [5,6]	Ornamental (aquaristics) and economic	Stabilized (has become invasive)
4.	*Gambusia holbrooki*	1927, Mangalia Lake [5,6]	Combating malaria	Stabilized
5.	*Lepomis gibbosus*	1929, Lower Danube Basin [5,6]	Natural pathways	Stabilized (has become invasive)
6.	*Ictalurusnebulosus*	1934, Tisa river [5,6]	Natural pathways	Stabilized (tending towards becoming invasive in Hungary)
7.	*Coregonus lavaretus*	1956, SCDP NucetDambovita; Tarcau River [4]	Aquaculture	Only in extensive aquaculture in mountain reservoirs
8.	*Coregonusalbula*	1956, SCDP NucetDambovita; Tarcau River [4]	Aquaculture	
9.	*Pseudorasbora parva*	1960, SCDP NucetDambovita [5,6]	Incidentally, together with the embryonated eggs of Asian cyprinid species used in aquaculture	Stabilized (has become invasive)
10.	*Ctenopharyngodon idella*	1960–1962, SCDP NucetDambovita [7]	Aquaculture	Stabilized
11.	*Mylopharyngodon piceus*	1960–1962, SCDP NucetDambovita [7]	Aquaculture	On fish farms and in Danube Delta

Table 1. *Cont.*

Crt. No.	Species	Date and Place of Introduction	Purpose of Introduction	Effects Produced and Reported
12.	*Hypophthalmichthys molitrix*	1960–1962, SCDP NucetDambovita [7]	Aquaculture	Stabilized
13.	*Aristichthys nobilis*	1960–1962, SCDP Nucet–Dambovita [7]	Aquaculture	Stabilized
14.	*Poecilia reticulata*	1975–1977, Oradea—1 Mai Lake	Ornamental	Disappeared
15.	*Ichthyobus cyprinellus*	1978, 1980, SCDP NucetDambovita [8]	Aquaculture	Disappeared
16.	*Ictiobus bubalus*	1978–1980, SCDP NucetDambovita [8]	Aquaculture	Disappeared
17.	*Ictiobus niger*	1978–1980, SCDP NucetDambovita [8]	Aquaculture	Disappeared
18.	*Iclalurus punctatus*	1980, SCDP NucetDambovita [8,10]	Aquaculture	Only in artificial ponds
19.	*Polyodon spathula*	1992, SCDP NucetDambovita; Acvares lasi	Aquaculture	In intensive aquaculture
20.	*Cyprinus carpio* var. KOI	1992, SCDP NucetDambovita, from Statiunea Piscicola Szarvasz, Budapest (Hungary)	Ornamental	-
21.	Hybrids of *Oncorhynchus mikiss* × *Salmo trutta fario*	2002–2003, Trout farm Oesti, Valsan River	Aquaculture	-
22.	*Mollietiisia sphenops*	1994, Oradea—1 Mai Lake	Ornamental	Disappeared
23.	*Xiphophonzs helleri*	1994, Oradea—1 Mai Lake	Ornamental	Disappeared
24.	*Clarias gariepinus*	1998–1999, Oradea	Intensive aquaculture	-
25.	Hybrids of *Acipenser* sp. × *Huso huso*	After 1998 in private farms, brought from Italy and Germany	Aquaculture	-
26.	*Percotus glennii*	2002, Dornesti Suceava and Siret River	Natural pathways	Stabilized and invasive in all Middle and Upper Danube
27.	Hybrids of *Oreochromis niloticus* × *Oreochromis mossambica*	2010	Aquaculture	-

Foreign fish species were introduced accidentally (small fish) or intentionally, for economic reasons or because of their aesthetic and landscape value. In accordance with the international perspective on alien species, our research aimed to determine the character or impact of each introduced species on native species in natural habitats or ecosystems of Romania, respectively, dividing them into two categories: non-invasive and invasive [11].

Non-invasive alien species are species that cannot proliferate in the wild without human help, so they cannot self-reproduce and do not show the ability to compete in food chains for the same resources as native species, and in a short and limited number of years, they disappear from the wild or decline due to competition with native species.

The situation is quite different for invasive alien species, with their direction of action being 180 degrees to that of non-invasive species. They have high invasiveness, marked by the following characteristics:

- Increased competition with native species for trophic and spatial resources, with invasive species out-competing native species;
- High prolificacy and the ability to survive and self-perpetuate in new habitats faster than native species;
- Manifestation of the tendency to eliminate competing native species for the same space and food resources, both through non-aggressive competition and through aggression itself, achieved through an appropriate competitive informational apparatus: chemicalhumoral (hormones and exohormones), territorial and aggressive behavior, evolved reproductive and offspring rearing behavior, result of a selective pressure from the natural range, complex and evolved social or group behavior, etc.

2. Experimental

Our research was carried out through experiments in National Institute for Research and Development in Environmental Protection (INCDPM Bucharest) Fish Research Laboratory (in 5 aquarium systems, equipped with centralized RAS type filtration), ethological and reproductive biology of captive fishes and research and observation of the behavior of fish species in the freshwater habitats of rheophilic and limnic freshwaters of Romania [12–16].

2.1. Materials

The 5 RAS aquarium systems were equipped with mechanical and biological filtration modules, aeration systems and lighting systems (with variable intensity and spectrum and with the possibility of realizing a photoperiod close to the natural environment).

Glass aquariums were used with the following dimensions: $\mathbf{S_1}$: 1200 × 600 × 600 mm (3 pcs), 1200 × 600 × 300 mm (3 pcs), 1200 × 600 × 300 mm (3 pcs) (Figure 1); $\mathbf{S_2}$: 1200 × 600 × 600 mm (4 pcs) (Figure 2a); $\mathbf{S_3}$: 1200 × 600 × 600 mm (5 pcs) (Figure 2b); $\mathbf{S_{XXL}}$: 3000 × 800 × 600 mm (1 pc) (extra-large aquarium) (Figure 3a); and $\mathbf{S_{Rh}}$: 3000 × 600 × 500 mm (1 pc) (rheophile aquarium) (Figure 3b).

The aquariums, being very large microsystems, were arranged in such a way as to reproduce as faithfully as possible the natural conditions of the limnic and rheophilic ecosystems of the Danube Basin. Typical plants for these ecosystems were used: *Potamogeton crispus*, *Polygonium amphibium*, *Nymphaea alba*, *Nuphar luteum*, *Hydrocharis morsus-ranae*, *Nymphoides peltata*, *Vallisneria spiralis*, *Salvinia natans*, etc. Also, original fauna was used: gastropods *Limnaea* sp., *Planorbis* sp. and *Melanopsis palustris* and shells *Anodonta cygnaea*. Willow and *Cyperaceae* roots and boulders were used as shelters to achieve greater heterogeneity of the biotope. The substrate used was variable, e.g., sand, gravel, boulders, and detritus, following the species preference for a particular type [17–21]. In laboratory conditions, the invasive species *Pseudorasbora parva*, *Lepomis gibbosus* and *Carassius auratus gibelio* were reared alone or in association with other species (Table 2).

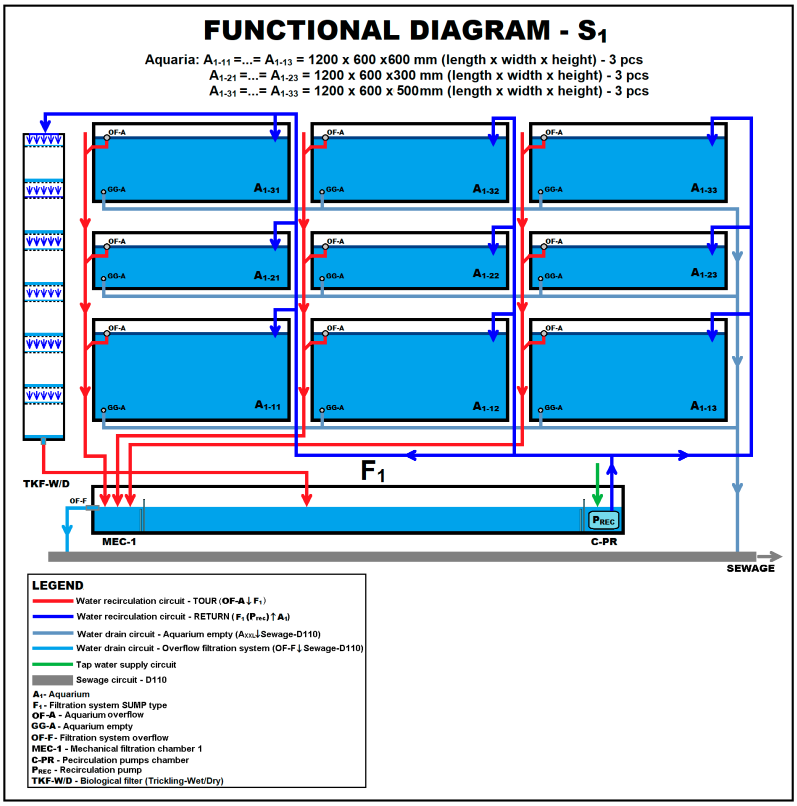
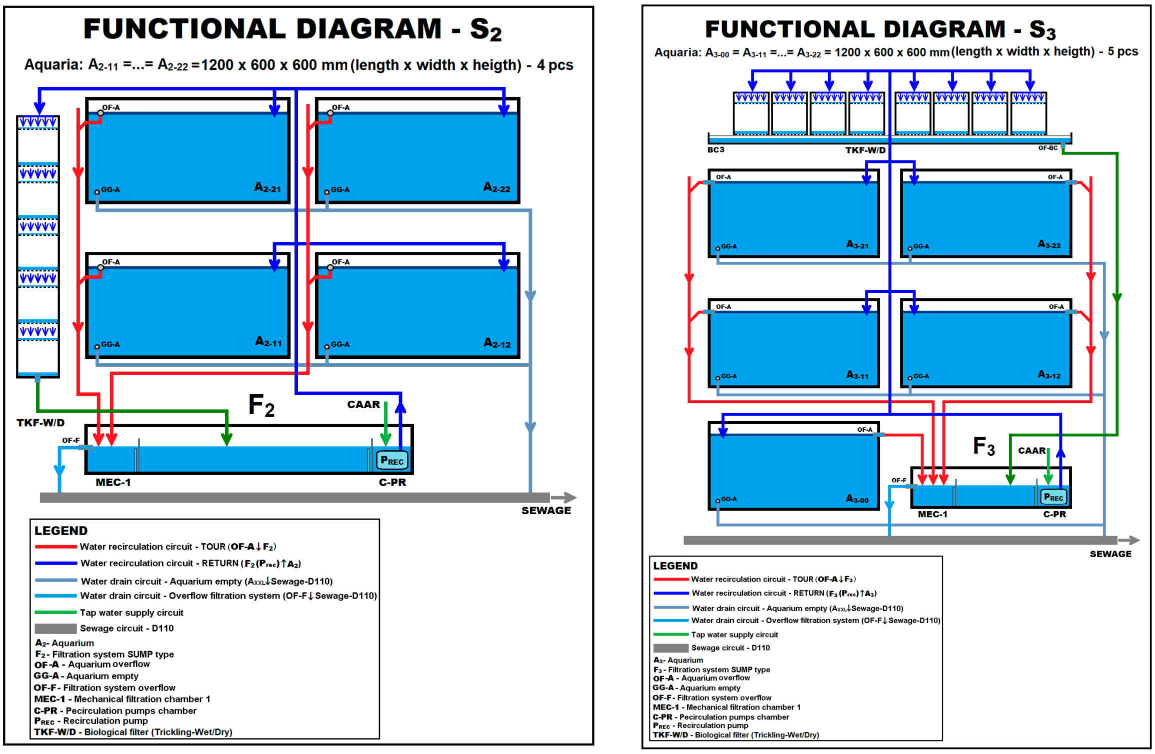
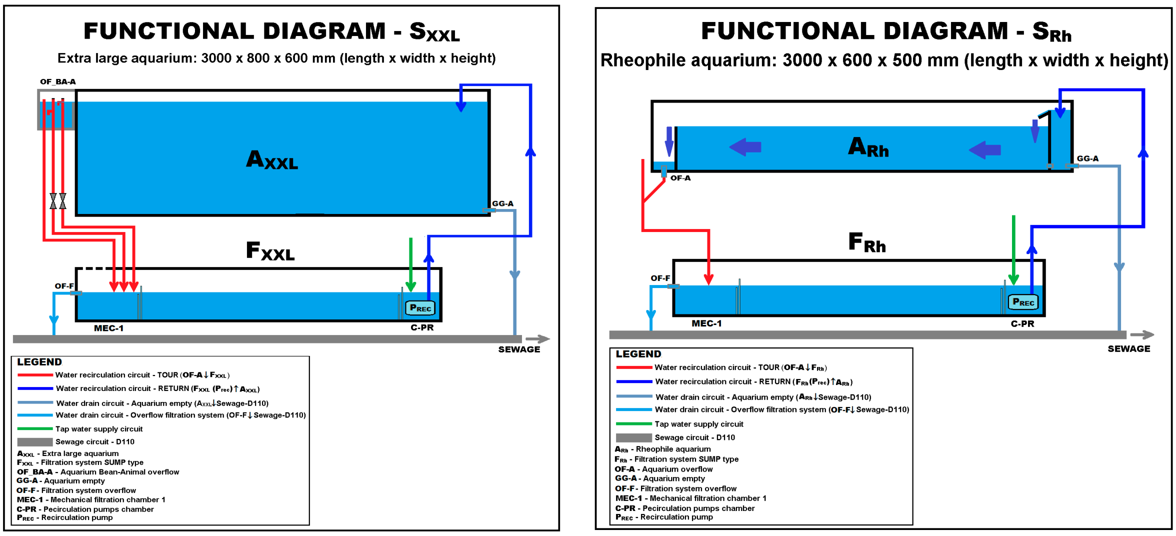


Figure 1. Experimental systems—functional diagram—$\mathbf{S_1}$.



(**a**) (**b**)

Figure 2. Experimental systems: (**a**) functional diagram—$\mathbf{S_2}$; and (**b**) functional diagram—$\mathbf{S_3}$.



(**a**) (**b**)

Figure 3. Experimental systems—extra-large aquariums: (**a**) functional diagram—$\mathbf{S_{XXL}}$; and (**b**) functional diagram—$\mathbf{S_{Rh}}$.

Table 2. Species associations used in aquarium systems experiments.

Experiment No.	Species Association		Experimental System Used	Aquariums Setup
	Species	No. of Individuals		
1	*Pseudorasbora parva* *Carassius auratus gibelio* *Cyprinus carpio*	Max, 25 Max, 25 Max, 25	S_1, S_2, S_3	Aquatic submerged plants: *Potamogeton crispus*, *Polygonium amphibium*, and *Vallisneria spiralis* Aquatic floating plants: *Nymphaea alba*, *Nuphar luteum*, *Hydrocharis morsus-ranae*, *Nymphoides peltata* *Salvinia natans* Original invertebrate fauna: gastropods *Limnaea* sp., *Planorbis* sp. and *Melanopsis palustris*; and shells *Anodonta cygnaea* Substrate: sand, gravel, boulders and detritus Shelters: willow and *Cyperaceae* roots
2	*Pseudorasbora parva* *Carassius auratus gibelio* *Lepomis gibbosus* *Cyprinus carpio*	Max, 20 Max, 20 Max, 20 Max,20	S_1, S_2, S_3	
3	*Pseudorasbora parva* *Carassius auratus gibelio* *Lepomis gibbosus* *Ctenopharyngodon idella* *Mylopharyngodon piceus* *Hypophthalmichthys molitrix* *Aristichthys nobilis*	50 50 20 10 5 10 5	S_{XXL}	
4	*Pseudorasbora parva* *Esox lucius*	100 10	S_{Rh}	
5	*Pseudorasbora parva* *Silurus glanis*	100 10	S_{Rh}	

2.2. Methods

During the scientific fishing activities, some of the biological material (fry, juveniles and adults) was brought to the INCDPM Bucharest Fish Research Laboratory, in the 5 aquarium systems described above. The fishing was carried out with two portable electrofishing devices with batteries: the Lena-Radomír Bednář (300 V/6 A, frequency: 55–95 Hz, pulse amplitude: 240–310 V, battery 12 V/7 Ahand Samus-725G (500 W, frequency: 2.5–99 Hz, 30 microsec.3.0 millisec., 12 V/7 Ah battery) [22].

For a more complete evaluation of the ichthyofaunal diversity, especially on the rivers, five rheophilic nets, modeled after Acad. Petru Banarescu, and a 7 m wide dragnet were used. They were placed during the fishing on the rivers at 3–4 m downstream of the electrofishing nets in order to train all the fish taken by the current in the nets, including benthic species, which are taken by the current following the electrofishing. Without the concomitant use of electrofishing gear and nets, it is not possible to realize a total scientific fishery on certain segments to emphasize all the species in the basin [11,23].

Observations were carried out daily, at 8:00–9:00 a.m. and 5:00–6:00 p.m., respectively, from an angle from which the fish were undisturbed and could display their typical behavior. Alternative procedures were also used: during feeding, cleaning of the aquarium, when turning the light on and off, or with the introduction of a new element (shelter) or new individual(s). The observation period in the 5 aquaria systems in the INCDPM Bucharest Fish Research Laboratory was 60 days.

3. Results and Discussion

Following the experiments of association in captivity of various species of fish, an attempt was made to reconstruct the situation existing in fish farms in the country at the time of the introduction of invasive fish species. In the experiments, within the studied species associations, the most common species farmed in extensive Romanian fish farming were used: *Cyprinus carpio*, the Asian cyprinids (*Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis* and *Mylopharyngodon piceus*), *Esox lucius* and *Silurus glanis*.

When setting up the experimental aquariums, we tried to reproduce as faithfully as possible the natural conditions in fish farms and natural lakes, related to substrate, physicochemical characteristics of the water (turbidity, lighting, pH, redox, conductivity and dissolved oxygen), water flow, vegetation shading, presence of aquatic invertebrates and amphibians, etc.

The associations between species in intensive aquaculture were analyzed from the ecological and ethological point of view in the fish farms of SCDP NucetDambovița, in order to evaluate the impact of invasive and non-invasive alien species on native fish species used in intensive and hyperintensive aquaculture.

Also, the experiments aimed to evaluate the effect of foreign invasive fish species on native species, reconstituting in the five aquaria systems in the INCDPM Bucharest Fish Research Laboratory the same associations that are realized in large aquaculture in fish farms, associations that we have scaled in Romanian aquaculture, as in nature in limnic and rheophilic habitats, evaluations made during the fishing, when live fish material was taken for the laboratory.

The following results were obtained:

I. In the associations made between *Pseudorasbora parva* and native peaceful species, the following was noted:
   - *Pseudorasbora parva* shows a competitive feeding behavior superior to native cyprinids that allows for rapid feeding. During feeding, a non-aggressive competitive behavior appears, with rapid zigzag swimming sequences or aggressive fins nipping behavior of other cyprinids or even percids (*Perca fluvialilis*) [11,24];
   - When food portions shrink, *Pseudorasbora parva* feeds on detritus, body mucus and fins of other cohabiting species. Under captive conditions, stress on the cohabiting native cyprinid species is induced [11];
   - *Pseudorasbora parva* originates from the genetic center of the Cyprinidae family and is the result of efficient selection carried out throughout evolution in the area with the highest selective pressure within the Cyprinidae family. As such, the species has developed mechanisms of interspecific competition and survival that

are adequate and extremely competitive—one could even say infallible— in the face of the indigenous European cyprinid species, which are practically disarmed in the face of this competitor;

- In our experiments in captivity we have discovered a very complex behavioral arsenal (defense and dissimulation behavior from predators manifested by a zigzag dance with short swimming sequences and short turns; shelter behavior realized in about 3–5 s with high speed of reaction to aggressors and specific and non-specific natural predators, and complex breeding behavior realized by group territorialism and choice of breeding areas guarded in common by several males, even if sexual selection is also manifested among them) [11,25].

II. *Carassius auratus gibelio* exhibits aggressive, competitive behavior that manifests itself especially during feeding, in captivity conditions in aquaria and in the ponds of SCDP NucetDambovita, chasing away from feeding sites *Cyprinus carpio*, *Aristichthys nobilis* and *Ctenopharyngodon idella* specimens by an aggressive zigzag dance with short aggressive zigzag swimming sequences [26].
Aggressive behavior has two phases [11]:

- Ritualized phase, with short zigzag dances of 2–3 s;
- Actual aggressive phase with interspecific fights by using the dorsal spine (first dorsal ray) in competition for food, in order to attack intruders from other species or even smaller conspecifics.

III. *Lepomis gibbosus* exhibits territorial and aggressive behavior very similar to species of the family Cichlidae [11,27]. The territoriality of the males is realized on territories with a radius of 15–25 cm, the intensity of the aggressive behavior being the highest in the center of the territory or in the place in the territory where there is a hollow shelter (rock and root). It was observed that nests were built at a distance of 30–40 cm from each other in captivity, in large aquaria of 2000 $\times$ 700 $\times$ 600 mm (about eight different territories) In natural environment, it was reported thousands of nests in the I.O.R Balta Alba Lake in Bucharest and Blasova Lake in the Small Island of Braila [11,27].

In captivity, the two invasive cyprinid species, *Carassius auratus gibelio* and *Pseudorasbora parva*, cohabit very well, as in the wild, the two species arecodominant in limnic and rheophilic habitats. There is maximum tolerance between the two species, as both species originate from Asia, where they are present in nature in the same habitats.

Lepomis gibbosus, together with *Pseudorasbora parva* and *Carassius auratus gibelio*, was found in the ponds of the Danube meadow at Braila (Filipoiu Channel), where the three species are dominant, even if they utilize different habitat, trophic and spatial resources. We do not know the cause of the association of the three species, but we assume that it is due to the increased invasiveness of all three species, due to the breach created in native ecosystems by their arrival and the increased competition with native fish species.

Even though invasive fish species come from different biogeographic regions (*Pseudorasbora parva* and *Carassius auratus* come from the Amur River basin in Asia, and *Lepomis gibbosus* from Central America and the Southern USA), they can cohabit and acquire new niches and spatial niches due to ecological imbalances in food webs or biochemical cycles in the habitats where they are present and have become invasive.

4. Conclusions

The disturbance of the ecological balance by pollution increases the invasiveness capacity of the three species mentioned above in parallel with the increased vulnerability of cohabiting native species in the aquatic ecosystems of the lower Danube Basin and large rivers of Romania.

In nature, invasiveness is determined by the degree of genetic homeostasis of aquatic ecosystems. For example, in the Gurbanului Valley rivulet, a tributary of the Neajlov River at Comana, even if the species *Pseudorasbora parva* is present, it is very rare along the entire 15 km long stretch of the rivulet, because the ichthyofaunal associations are very diverse, being composed of 18 freshwater fish species in association with thousands of phytozoobenthic and phytozooplanktonic species of hydrobionts.

Pseudorasbora parva has been found in many types of freshwater habitats, from the Danube Delta to the hilly area at altitudes of 600–700 m in the ichthyological zone of the *Chondrostoma nasus*; on the Valsan river and even in the area of the *Thymallus thymallus* and the *Barbus petenyi*, the Dambovita and Doamnei Rivers.

Carassius auratus gibelio is dominant in limnic habitats with strong mineralization and temporary nocturnal lack of oxygen (zamor phenomenon) in waters with algal eutrophication by cyanobacteria and thiobacteria, dinoflagellates, etc., and lack of self-control mechanisms of eutrophicated and degraded ecosystems of the plain. Such habitats are found in the vicinity of settlements and industrial areas, where economic agents are sources of pollution with the highest possible ecological impact.

Carassius auratus gibelio showed an aggressive behavior towards all the cyprinids in the experiments, a competitive behavior clearly superior to the other species, giving it increased advantages in survival and natural selection. This behavior inhibits the feeding of all other cyprinid species in the tank, except for the predatory species (*Leuciscus cephallus*) or predatory species of other families (*Lepomis gibbosus*).

In limnic ecosystems, both natural and artificial (fish farms), there is very strong interspecific competition between *Cyprinus* carpio and Carassius *auratus auratus gibelio.* Under captive conditions, stress factors are eliminated as much as possible, and the fish are placed in very large aquaria and in experimental formula: *Carassius auratus gibelio* × *Cyprinus carpio* × *Pseudorasbora parva*. Also, an aggressive behavior of the carassius *auratus gibelio* toward carp was observed.

It is possible that the new Asian cyprinids introduced intentionally or escaped in the wild (*Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis* and *Mylopharyngodon piceus*) will behave in the next 50 years like *Carassius auratus gibelio*, leading to the replacement or disappearance of native cyprinid species in limneous or rheophilic ecosystems. The first targets would be *Scardinius erithrophthalmus*, *Rutilus rutilus*, genus *Abramis*, *Pelecus cultratus*, etc., i.e., cyprinids with close ecological niches.

Lepomis gibbosus is present in the degraded riparian ecosystems between the reservoirs of the main rivers. The contribution of this species to the riparian habitats is determined by the surplus of individuals reproducing in the lakes and not finding sufficient food, which is why they micromigrate in the riparian sectors upstream of the lakes.

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Conflicts of Interest: The authors declare no conflicts of interest.

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