

The effect of fish and aquatic habitat complexity on amphibians

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Abstract Fish introductions are considered one of the most widespread anthropogenic threats to aquatic ecosystems. Their negative impact on native amphibian communities has received increasing attention in recent years. We investigated the relationship between the introduced fish, emergent vegetation cover and native amphibians in man-made ponds generated by regulation and dam building along the Târnava Mare Valley (Romania) during the last 40 years. We inventoried amphibians and fish inhabiting 85 permanent ponds and estimated habitat complex-

ity focusing on emergent vegetation cover. Four amphibian species were found to be negatively associated with the presence of predatory fish. Species richness of ponds without fish and ponds without predatory fish did not differ significantly, whereas ponds containing only predatory fish had significantly lower amphibian richness. A significant positive relationship was found between the emergent vegetation cover and pond occupancy of six amphibian species and amphibian species richness. As a management recommendation, we suggest the restriction of fish introductions to non

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AUTHOR'S PROOF!

predatory fish and the maintenance of high emergent vegetation cover in the ponds.

Keywords Introduced fish · Emergent aquatic vegetation cover · Amphibian conservation · Romania

Introduction

Fish introductions are considered one of the most widespread anthropogenic threats to aquatic ecosystems and native species (Chapman et al., 1996; Lodge et al., 1998; Schindler et al., 2001; Olden & Poff, 2005) and represent one of the major causes in the decline of amphibians worldwide (Kats & Ferrer, 2003). Water breeding amphibians are sensitive to fish introductions through their aquatic stages (eggs, larvae, breeding adults and adults that remain in the water for feeding). Introduced fish can reduce or completely eliminate amphibians throughout predation, competition and even pathogen transfer (Brönmark & Edenhamn, 1994; Hecnar & M'Closkey, 1997; Tyler et al., 1998; Knapp & Matthews, 2000; Kiesecker et al., 2001; Matthews et al., 2001; Larson & Hoffman, 2002; Nyström et al., 2002; Kats & Ferrer, 2003; Bosch et al., 2006; Orizaola & Brana, 2006). Introduced fish are responsible for the widespread extinction of paedomorphic newt populations in Europe (*Triturus alpestris* and *T. helveticus*) (Denoël et al., 2005). However, different species from the same amphibian community may show different sensitivities to changes in habitat quality, including fish predation (Hecnar & M'Closkey, 1997).

The permanent aquatic habitats represented by ponds in lowland and hilly areas may have different origins (although frequently both are seminatural), their formation being the result of increasing hydroperiod of temporary ponds, river regulation, dam building, creation of garden ponds etc. In cases where the permanent ponds originate through increasing hydroperiod of temporary ponds, the establishment of new and/or alien fish species can be detrimental for those amphibians that are adapted to reproduce in these ponds (Maret et al., 2006). If the ponds are the result of river regulation, the amphibian commu-

nity may be historically exposed to various fish predators (fish-amphibian communities of flooded areas [Pintar & Spolwind, 1998]). This could result in a co-evolutionary adaptation of amphibians to fish predators in order to decrease predation risk, whereby varying habitat complexity and the hydrodynamic gradient might play an important role (Real et al., 1993; Pintar & Spolwind, 1998; de Nooij et al., 2006). The permanent aquatic habitats from the low elevation areas are often vegetated with both emergent and submerged vegetation. Vegetation in ponds may create a variety of microhabitats (Laan & Verboom, 1990). Littoral habitat complexity (in terms of vegetation cover) plays an important role as refuge habitat against predation in pond predator-prey systems (Holopainen et al., 1997; Sass et al., 2006). These highly productive microhabitats may represent safe and food rich environments for amphibian larvae and aquatic adults, and provide good quality microhabitats for reproduction (being support for eggs) (Hartel, 2004).

Fish introductions in Romania have become more frequent and popular, as the ponds have moved into private ownership. These introductions, in combination with other anthropogenic impacts (i.e. reduction of macrophyte cover) and with changes to the surrounding terrestrial habitats are expected to have a negative impact on amphibians in this region. Therefore we need baseline datasets regarding both habitat characteristics and species composition for making better decisions on amphibian conservation.

In this paper we relate the occurrence of fish with the presence of amphibians in a hilly area of Central Romania. The objectives of this paper are: (1) to analyse the potential impact of the introduced fish on the pond occupancy of individual amphibian species and amphibian species richness, and (2) to analyse the importance of habitat complexity for individual species and species richness of amphibians.

Materials and methods

Study area and data collection

The study area covers approximately 2,600 km², and is situated in the middle section of the

Târnava Mare Valley, Romania. The regulations of the river and dam building along its tributaries during the last 40 years have generated a number of ponds along the valley. The fish introduced into the new ponds included predatory species, some of these native to the Romanian fauna that were absent in the past from the Târnava Mare Valley (e.g. *Perca fluviatilis*, *Stizostedion lucioperca*, *Silurus glanis*, *Esox lucius*) and two alien species (*Lepomis gibbosus*, *Pseudorasbora parva*) (Bănărescu, 1964; Wilhelm, 2000).

The ponds were localized using 1:25,000 scale topographic maps, information provided by the landowners and active search. All inventoried ponds were located using a handheld Global Position System (GPS). The amphibian surveys were made between March and August each year. The ponds were surveyed during 2000–2005 period: 23 ponds were regularly surveyed in 3–5 years in this period, another 42 ponds were surveyed in 2004 and an additional 20 ponds were surveyed only in 2005. In 2005, 12 ponds first located in 2004 were resurveyed. Amphibians were inventoried by searching for eggs, dipnetting (for adults and larvae), torch counts and the detection of calling anuran males. Two to three surveys were made on each pond during the amphibian's reproductive period (March until mid-May), and another two to three surveys were carried out on each pond until the end of July for larvae.

Many studies on the relationship between amphibians and fish use presence/absence data for fish in different ponds (see for example Hecnar & M'Closkey, 1997; Ficetola & De Buernardi, 2004; Orizaola & Brana, 2006). We determined the presence of fish through visual observations, dipnetting and information gathered from fishermen, pond owners and fishing agencies (similar methods to estimate fish presence and absence being frequently used in such kind of studies, see Hecnar & M'Closkey, 1997; Baker & Halliday, 1999). Visual observations and dipnetting were used in every pond to detect the presence/absence of fish species. These methods were found to be efficient in detecting *Lepomis gibbosus*, *Perca fluviatilis*, *Pseudorasbora parva* and *Squalius cephalus* but not for the detection of the larger bodied predatory fishes, such as *Silurus glanis*, *Esox lucius*, *Stizostedion lucioperca* and

other large bodied non predatory fishes (see below). Informations from landowners (if the ponds were privately owned) and fishing agencies were also considered in the case of every pond (for all fish species) and completed with informations gathered by fish men. The fish species were grouped in two categories: non-predatory fish (*Carassius auratus*, *Cyprinus carpio*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Leucaspis delineatus*, *Scardinius erythrophthalmus*) and predatory fish (*Esox lucius*, *Squalius cephalus*, *Lepomis gibbosus*, *Perca fluviatilis*, *Pseudorasbora parva*, *Silurus glanis*, *Stizostedion lucioperca*, *Salmo fario*).

The following criteria were used when grouping fish species in these categories: their feeding biology in Romania (Bănărescu, 1964; Wilhelm 2000), experimental observation reports about their harm effect on the different life stages of amphibians (see Teplitsky et al., 2003 for the species *Pseudorasbora parva*) field observations (see Brönmark & Edenhamn, 1994) and reviews (see Gillespie & Hero, 1999; Kats & Ferrer, 2003). The goldfish (*Carassius auratus*) was included in the "non-predatory" fish category in this study, although there are field observations about their negative impact on *Rana temporaria* populations (see Meyer et al., 1998). In our study area however, the goldfish is widely introduced and long-term studies show that the amphibian populations are not affected by this fish species (Hartel, 2004). The ponds were afterwards classified into three categories: ponds without fish, fish ponds without predatory fish and ponds with predatory fish.

The aquatic habitat complexity was estimated visually, as the percentage cover of emergent aquatic vegetation (mainly *Phragmites* sp. and occasionally *Typha* sp.) in each pond, two observers did this independently. The emergent vegetation cover was estimated once in spring, during the breeding period of amphibians. In the analysis we used the values of the estimated emergent vegetation cover from the year in which the last amphibian survey was made in a particular pond. Since in early spring, during the breeding period of amphibians, only the emergent vegetation is present (Hartel, 2004), submergent vegetation that developed afterwards (e.g. *Myriophyllum* sp., *Ceratophyllum* sp.) was not taken into account.

Statistics

Presence/absence data gathered during the years were pooled for statistical analysis. The incidence of amphibian species was recorded as a 0/1 binary variable y . One continuous (emergent vegetation cover) and two discrete binary variables (the presence or absence of predatory and non-predatory fish) were used as predictors in a Binary Response Model using logistic regression (Long, 1997). Non-significant terms were stepwise removed and the final model contained only significant terms. Assessment of model performance was made by the Area Under the Receiver Operating Characteristic Curve (AUC). AUC is a powerful, threshold-independent measure of overall fit that varies between 0.5 (for a chance performance) to 1.0 for a perfect fit (Fielding & Bell, 1997; Jesús & Ángel, 2004).

A one-way ANOVA and subsequent Fisher LSD test was run to compare amphibian species richness and the emergent vegetation cover among the three pond categories. The relationship between amphibian species richness and emergent vegetation cover, presence of non-predatory fish and presence of predatory fish was analysed using generalised linear models (GLM). As species richness data were counts, a Poisson error distribution with a log link function was used (Quinn & Keough, 2002).

Results

Ten species of amphibians and a species complex were found: *Rana dalmatina* (overall pond occupancy 84%), *Bufo bufo* (81%), *R. esculenta* complex (81.1%), *Hyla arborea* (72%), *R. temporaria* (57%), *Triturus cristatus* (51%), *Bombina variegata* (46%), *T. vulgaris* (41%), *Pelobates fuscus* (26%), *B. viridis* (6%) and *R. arvalis* (1%). Figure 1 shows the pond occupancy of amphibians in the three pond categories.

Ponds without fish represented 30% of all ponds, fish ponds without predatory fish 41.17% and ponds with predatory fish 29%. All the ponds that contained predatory fish also contained non-predatory fish. *Perca fluviatilis* had the highest percent occurrence followed by *Pseudorasbora parva* and *Silurus glanis* (Table 1).

Emergent vegetation cover was significantly different between the three pond categories (ANOVA $F_{[2, 81]} = 11.68$, $P < 0.0001$) the ponds without fish having a significantly greater cover than the ponds without predatory fish (average 55.8, SD = 40.1 vs 34, SD = 20) ($P < 0.001$) and ponds with predatory fish (average 23.04, SD = 23) ($p < 0.001$). The emergent vegetation cover does not differ significantly between the ponds without predatory fish and ponds containing predatory fish ($P = 0.80$) (Fisher LSD; $MS = 451.43$, $df = 32$).

Fig. 1 The proportion of ponds without fish, fishponds without predatory fish and ponds with predatory fish occupied by different amphibian species in Târnava Mare Valley

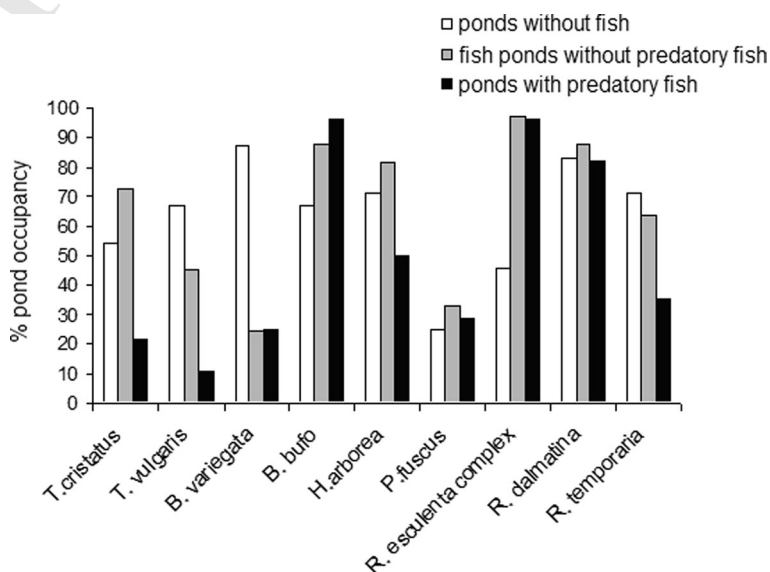


Table 1 The percentage of occurrence of the predatory fish species in all ponds and in ponds containing predatory fish and in the set of ponds that also contain non-predatory fish

Fish species	Overall percentage (<i>n</i> = 85 ponds)	Percentage in ponds with predatory fish (<i>n</i> = 25 ponds)
<i>Perca fluviatilis</i>	16.47	56.00
<i>Pseudorasbora parva</i>	16.47	56.00
<i>Silurus glanis</i>	14.11	48.00
<i>Lepomis gibbosus</i>	9.41	32.00
<i>Stizosteidon lucioperca</i>	8.23	28.00
<i>Squalius cephalus</i>	5.88	20.00
<i>Esox lucius</i>	4.70	16.00
<i>Salmo fario</i>	1.17	4.00

Single species analysis

Single species models relating incidence to emergent vegetation cover, predatory fish and non-predatory fish presence showed overall high model accuracy (AUC ranges from 0.74 to 0.87) except for

P. fuscus (AUC = 0.69; Table 2). The occurrence probability of six species was positively associated with emergent vegetation cover of which four species were negatively affected by the presence of predatory fish (Table 2). Four species (*B. variegata*, *B. bufo*, *R. esculenta* complex, and *R. dalmatina*) were seemingly unaffected by the presence of the predatory fish while *T. cristatus*, *B. bufo* and *R. esculenta* complex were positively associated with non-predatory fish (Table 2).

Species richness

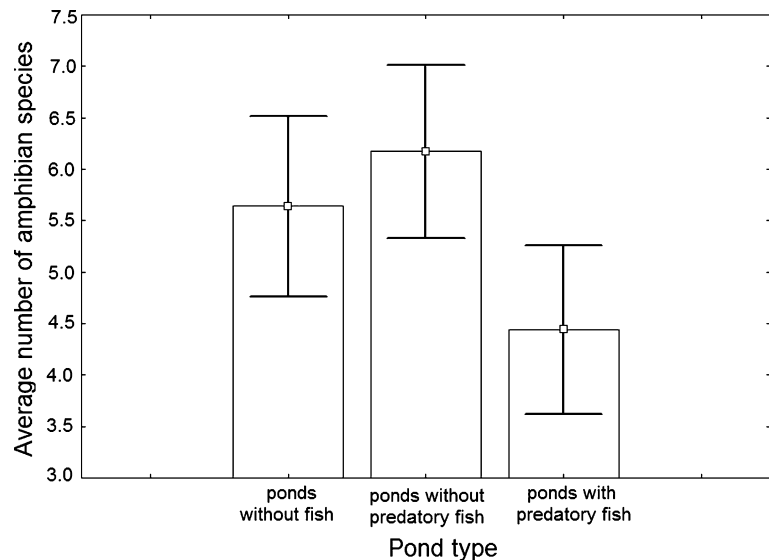
Amphibian species richness significantly differed among the three pond categories (ANOVA, $F_{[2, 81]} = 4.04$, $P < 0.05$). A post-hoc comparison (Fisher LSD, $MS = 4.83$, $df = 81$, $P > 0.05$) showed no difference between the ponds without and the fish ponds without predatory fish. However, species richness in both of these pond types was significantly larger than in the predatory fish ponds (both $P < 0.05$) (Fig. 2).

Table 2 Summary of the logistic regression analysis of the relationship between the fish and reed cover and amphibian pond occupancy

Species	Variable	$\beta \pm 1SE$	Wald Z	P-value	R ²	AUC
<i>Triturus cristatus</i>	PF	-2.45 ± 0.66	-3.69	<0.001	0.56	0.86
	NPF	2.54 ± 0.9	2.83	0.005		
	EV	0.056 ± 0.014	3.81	<0.0001		
<i>Triturus vulgaris</i>	PF	-1.93 ± 0.72	-2.67	0.008	0.63	0.81
	NPF	-0.120 ± 0.63	-0.19	NS		
	EV	0.032 ± 0.01	2.83	0.005		
<i>Bombina variegata</i>	PF	-0.476 ± 0.64	-0.74	NS	0.63	0.87
	NPF	-2.95 ± 0.8	-3.47	0.001		
	EV	0.041 ± 0.01	2.79	0.005		
<i>Bufo bufo</i>	PF	0.862 ± 0.89	0.97	NS	0.62	0.76
	NPF	1.68 ± 0.74	2.27	0.02		
	EV	0.018 ± 0.01	1.79	NS		
<i>Hyla arborea</i>	PF	-0.701 ± 0.65	-1.08	NS	0.39	0.79
	NPF	0.525 ± 0.79	0.67	NS		
	EV	0.044 ± 0.013	3.26	0.001		
<i>Rana esculenta</i> complex	PF	0.420 ± 1.26	0.33	NS	0.67	0.87
	NPF	3.93 ± 1.94	3.43	0.01		
	EV	0.004 ± 0.01	0.39	NS		
<i>Rana dalmatina</i>	PF	0.225 ± 0.87	0.26	NS	0.61	0.87
	NPF	0.660 ± 0.94	0.7	NS		
	EV	0.101 ± 0.03	3.23	0.003		
<i>Rana temporaria</i>	PF	-0.770 ± 0.56	-1.37	NS	0.29	0.74
	NPF	0.593 ± 0.62	0.95	NS		
	EV	0.031 ± 0.01	2.73	0.003		
<i>Pelobates fuscus</i>	P	-0.168 ± 0.61	-0.28	NS	0.28	0.69
	NPF	1.076 ± 0.73	1.47	NS		
	EV	0.017 ± 0.01	1.59	NS		

PF, predatory fish; NPF, non-predatory fish; EV, emergent vegetation cover; NS, not significant

Fig. 2 Histogram for the average number of amphibian species in the three pond types in Târnava Mare Valley together with error bars representing the 95% confidence interval for the mean



The amphibian species richness was significantly positively associated with the amount of emergent vegetation cover and the presence of non predatory fish, while a negative relationship was found between the amphibian species richness and the presence of predatory fish (Table 3).

Discussion

Negative effects of fish introduction on European amphibian species such as the Iberian frogs (*Rana iberica*) (Bosch et al., 2006), moor frog (*Rana arvalis*) (Sas et al., 2006), spadefoot toad (*Pelobates fuscus*) (Nyström et al., 2002), common tree frog, (*Hyla arborea*) (Brönmark & Edenhamn, 1994) or urodelans (*Triturus helveticus*, *T. alpestris*, *T. cristatus*, *T. marmoratus*, *Salamandra salamandra*) (Joly et al., 2001; Martinez-Solano et al., 2003; Reshetnikov, 2003; Denöel et al., 2005; Orizaola & Brana, 2006; Skei et al., 2006)

have recently been reported. Our results confirm the negative impact of predatory fish on individual amphibian species and overall species richness in the field, and also provide evidence for the positive relationship between the amphibian species and high levels of emergent vegetation cover and with the presence of non-predatory fish in the case of some species.

The negative effect of fish predators on amphibians in this area could be manifested through direct predation and competition for food. The small sized predatory fish such are *P. parva*, *L. gibbosus*, *P. fluviatilis* cannot prey on adult amphibians because of gape limitation but frequently eat their eggs and larvae or cause severe injuries in amphibian larvae (Sas, I., Hartel, T., personal observations in the field). The large sized predatory fish (*Silurus glanis*, *Squalius cephalus*, *Esox. lucius*, *Stizosteidon lucioperca*, *Salmo fario*) can consume even the postmetamorphic stages of amphibians (Hartel, T. personal observation). The nektonic characteristic (the fact that they move in the open water) of

Table 3 Results of a logistic regression analysis of amphibian species richness in function of emergent vegetation cover, the incidence of non-predatory fish, and the incidence of predatory fish

Predictor	$\beta \pm 1SE$	Wald χ^2	P-value	L95%CI	U95%CI
Predatory fish	-0.265 \pm 0.113	5.45	0.01	-0.489	-0.042
Non-predatory fish	0.342 \pm 0.131	6.78	0.009	0.084	0.60
Emergent vegetation cover	0.009 \pm 0.001	22.51	<0.0001	0.005	0.012

SE, standard error; L95%CI, lower 95% confidence interval; U95%CI, upper 95% confidence interval

some amphibian larvae (*T. cristatus*, *H. arborea*) also may expose them to predation by the visually oriented fish predators (ex.: *L. gibbosus*, *P. fluviatilis*, *E. lucius*) (Manteifel & Reshetnikov, 2002). Tadpoles of common toad (*B. bufo*) are unpalatable to fish due to their toxic secretions (Manteifel & Reshetnikov, 2002; Crossland & Alford, 1998). Beside direct predation, introduced fish negatively affect the abundance of aquatic invertebrates (such are crustaceans, oligochaetes and chironomids) (Berg et al., 1994; Reshetnikov, 2003) that may constitute a major part of the prey of newts (*T. vulgaris* and *T. cristatus*) (Joly & Giacoma, 1992).

Experimental studies show that the larvae of *H. arborea*, *R. temporaria*, *R. dalmatina*, *R. esculenta* complex and newts from the genus *Triturus* display morphological and behavioural responses in the presence of predators (Semlitsch & Reyer, 1992; Stauffer & Semlitsch, 1993; Laurilä, 2000; Van Buskirk & Schmidt, 2000; Van Buskirk, 2002; Teplitsky et al., 2003) that may increase survival until metamorphosis under predation pressure. Some of these species (*H. arborea*, *R. temporaria*, *T. cristatus* and *T. vulgaris*) are not able to coexist with predatory fish in the field (Brönmark & Edenhamn, 1994; Meyer et al., 1998; Baker & Halliday, 1999; Joly et al., 2001; Orizaola & Brana, 2006; Skei et al., 2006; this study) whereas others (*R. dalmatina*, *R. esculenta* complex) seem to be unaffected by fish (our study).

Field observations show that ponds may become inhospitable habitats for amphibians (*H. arborea*, newts from North America) in only a short time period following the introduction of predatory fish (in a one to two year period after the introduction of the fish [Brönmark & Edenhamn, 1994; Hecnar & M'Closkey, 1997]). However, rapid recoveries of breeding habitat quality after fish removal were also reported: in Sweden, *Hyla arborea* successfully reproduced in a pond the spring after the recent removal of fish (tench, roach and perch) (Brönmark & Edenhamn, 1994). Similar results were found after the removal of salmonids from mountain ponds (Hoffman et al., 2004; Vredenburg, 2004). In our study area we monitored for four years two ponds that contained high densities of *S. glanis*, *P. fluviatilis*, *P. parva* and also *E. lucius*.

The two newt species (*T. cristatus* and *T. vulgaris*), and the tree frog (*H. arborea*) were found to be absent from these ponds indicating that these ponds are inhospitable habitats for these species. However, all three species were found in high abundances in a shallow, vegetated pond containing non-predatory fish (*Leucaspis delineatus*) at a distance of about 500 m from the previous ponds (Hartel, unpublished data).

Predatory fish may also have a positive indirect effect on some amphibians due to differential predation on the on their competitors and predators, like dragonfly larvae (Smith et al., 1999; Maezono & Miyashita, 2003). This is likely the case when amphibians are unpalatable to fish predators (Smith et al., 1999). In our case *B. bufo* may benefit from fish introductions, since common toad larvae are unpalatable to fish.

Our study highlights the importance of the structural complexity of the aquatic habitats, quantified by the emergent vegetation cover. The pond occupancy of six amphibian species was significantly positively associated with the emergent vegetation cover, so as the species richness. Similarly, Hecnar & M'Closkey (1997) found a significant positive relationship between the emergent vegetation cover and the amphibian species richness in ponds containing predatory fish. Emergent vegetation grows in the littoral, shallow productive part of the ponds and offers support for eggs, shelter and food for amphibian adults and larvae. Vegetation cover may also have a role as a defence against predation.

The productive and highly structured littoral zones of the ponds were found to be important habitat refuge for curcian carp (*Carassius carassius*) juveniles in large lakes where their populations are regulated by predation from piscivores (perch, pike) (Holopainen et al., 1997). In a structurally complex habitat, the foraging efficiency of predators is reduced (Babbitt & Tanner, 1997; Manatunge et al., 2000). Tarr & Babbitt (2002) showed that the survival of *R. clamitans* tadpoles was significantly correlated with vegetation density: in the absence of vegetation cover the survival of tadpoles was very low when exposed to invertebrate predators. With increasing vegetation cover the survival of the tadpoles increased. Joly et al. (2001) found that aquatic

vegetation was the primary pond variable that influenced the abundance of *T. helveticus* and *T. cristatus*. A long-term study on a *R. dalmatina* population in the Târnava Mare Valley using egg mass counts showed that microhabitat preference for spawning was influenced by the distribution and succession stage of the emergent vegetation, particularly reed (Hartel, 2004).

Fish introductions tend to be more and more frequent in this area as the ponds are coming into private ownership. It is expected that the habitats used by amphibians will become increasingly exposed to negative anthropogenic pressure in the future (more fish introductions, reduction of vegetation cover, modifications in the surrounding landscape). To efficiently protect amphibians, a strong collaboration between landowners, landscape managers and scientists is needed. As a management recommendation, we suggest the restriction of fish introductions to non-predatory fish ponds and the maintenance of high emergent vegetation cover in the ponds. We also recommend the maintenance of ponds where no fish are introduced at all. This will contribute to among pond and gamma diversity of amphibians in the region. Besides the positive effect on amphibians, a larger emergent vegetation cover could be beneficial to many other vertebrate species, including non predatory fish and birds through assuring habitat for breeding and nesting.

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