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Effects of introduced salmonids on amphibian distribution and abundance in Retezat National Park

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Abstract

A total of 105 ponds and lakes across the alpine area of Retezat National Park were surveyed in order to find if the introductions of nonnative fish species have a negative influence on the distribution and abundance of local amphibian populations. Surveys conducted over two summer months in 2006 were used to identify the lakes where the introduced fish species still occur and to note the status of amphibian populations. Experiments performed at two sites revealed that the fish species found in the area are capable of heavily preying on and drastically reducing the number of tadpoles. The results of the study also show that the remaining amphibian populations are very fragmented and missing from several sites that were stocked with fish which have later disappeared. Restoration of amphibian populations in some of the lakes is required and a good long term monitoring program should be set up to support the amphibian recovery process.

Keywords: amphibians, introduced species, fish, population decline, predation, invasive alien species, *Rana temporaria*, *Mesotriton alpestris*

Contents

Introduction

Worldwide many amphibian populations have shown a declining trend (Bradford et al. 1998, Gillespie 2001, Knapp & Matthews 2000b). There are many factors involved in this decline such as global warming, habitat destruction, the use of pesticides and herbicides and the introduction of predators (Denoel et al 2005, Hecnar & M'Closkey 1997, Knapp & Matthews 2000b). The most commonly introduced predators affecting amphibians are a number of fish species. Fish have mainly been introduced as biological control agents, especially for the control of mosquitoes (Lawler et al. 1999, Young and Harrig 2001), and to improve recreational fisheries (Bank et al. 2006, Bradford et al. 1998, Knapp & Matthews 2000a, Pilliod & Peterson 2000). For recreational purposes, many cold water species, mainly salmonids, have been widely introduced in lakes and rivers around the world, with substantial effects on local amphibian communities (Bradford 1989, Brönmark & Edenhamn 1994, Gillespie 2001, Hecnar & M'Closkey 1997, Knapp & Matthews 2000b, Denoel et al. 2005).

Amphibians may be affected by the introduced fish predators in two general ways, directly and indirectly. Direct predation simply means that one or several life stages of the amphibians are being eaten by the fish. In many cases the earlier life stages are the most vulnerable (Bradford et al. 1998, Gillespie 2001, Knapp & Matthews 2000b). It has been shown that some amphibian populations have disappeared locally because of direct fish predation (Bradford 1989). There is a clear relationship between amphibian body size and vulnerability to predation. The smaller the size, the higher the predation level is (Schmidt & van Buskirk 2005), but in the same time different life stages may be vulnerable to different predators.

Introduced fish may also give an indirect effect, for example by reducing the number of places where amphibians can breed, or by changing the behaviour of the amphibians. Tadpoles have been shown to reduce their activity and spend more time in shelter to avoid predation when fish predators are present (Orizaola & Brana 2003). Some amphibian tadpoles have also shown a reduction in their activity even when other types of predators such as dragonfly Odonata larvae and backswimmer Notonectidae adults were present (Laurila 2000, Van Buskirk 2001). Furthermore, they significantly decrease their activity when predator chemical cues are detected (Bosch et al. 2005), there is a reduction in the time they need to reach metamorphosis, they are smaller at metamorphosis and grow faster after metamorphosis. Such changes in the earlier life stages might be associated with changes to demographic performance in later stages (Orizaola & Brana 2005).

The effects of introduced predatory fish are expected to be more severe in mountain regions, for several reasons. The isolation of lakes and ponds is one factor, as the distance between waters suitable for breeding may be much longer than the amphibians' dispersal capacity (Bradford et al. 1993, Tyler et al. 1998). Dispersal may also be physically hindered by natural barriers such as ridges and precipices. Once the fishes have eaten the tadpoles from one lake, there is a limited probability that adults can move to other places to breed in the second year due to limitations in access. Another problem in alpine environments is related to the very short ice-free period during which the amphibians can breed (Knapp & Matthews 2000b, Tyler et al. 1998).

In addition to the above described indirect effects of introduced fish species on amphibians comes the introduction of diseases in lakes through different pathogens and parasites. This may happen even when the fishes are reared in hatcheries (Blaustein et al. 1994). The most widespread disease transmitted from fishes to amphibians is the water mold *Saprolegnia ferax*, which is the main cause of egg mortality in the amphibian populations (Blaustein et al. 1994). Fish species in the genera *Salmo*, *Salvelinus* and *Oncorhynchus* are common carriers of water mold (Dunham et al. 2004). The immediate effect following stocking is that the infected fish might directly transmit the water mold to developing amphibians, but it can also be transferred to the lake substrate where it may become established (Kiesecker et al. 2001).

There is a general opinion that amphibian populations in the alpine areas of Romania have declined and even disappeared from some of the areas because the tadpoles survival is extremely low (Cogalniceanu et al. 2001). Several species of salmonids have been introduced in the alpine lakes. They are still present in some of the lakes and have disappeared from others (where they have been present for some time) (Decei 1981).

The question is whether there is a connection between fish introductions and loss of amphibians in the studied lakes and ponds lakes. Is the trout in particular responsible for the amphibian decline and loss of breeding sites? A related question is whether it is the fish populations that are still present in the area that were responsible for the reduction in amphibian distribution, or whether fish populations that are now extinct had an even larger effect in this reduction? Is there any difference in the predation level between the different fish species? Do the different amphibian species react in different ways to the fish introduction? Which are the most affected amphibian life stages? Do the adult amphibians avoid breeding in the lakes with fish population even though they may use these lakes in the later life stages?

Study area and species

The study area (Fig.1) is located in the oldest Romanian national park, Retezat, which was established in 1935 in the Southern Carpathian Mountains. The national park measures 38 000 ha. The study area was limited to the alpine zone, excluding the Gemenele scientific reserve which has very restricted and controlled access. The lakes in the study area are found in six catchments, Raul mare, Nucsoara, Rau Barbat, Jiul de Vest, Vasielului valley and Stevia.

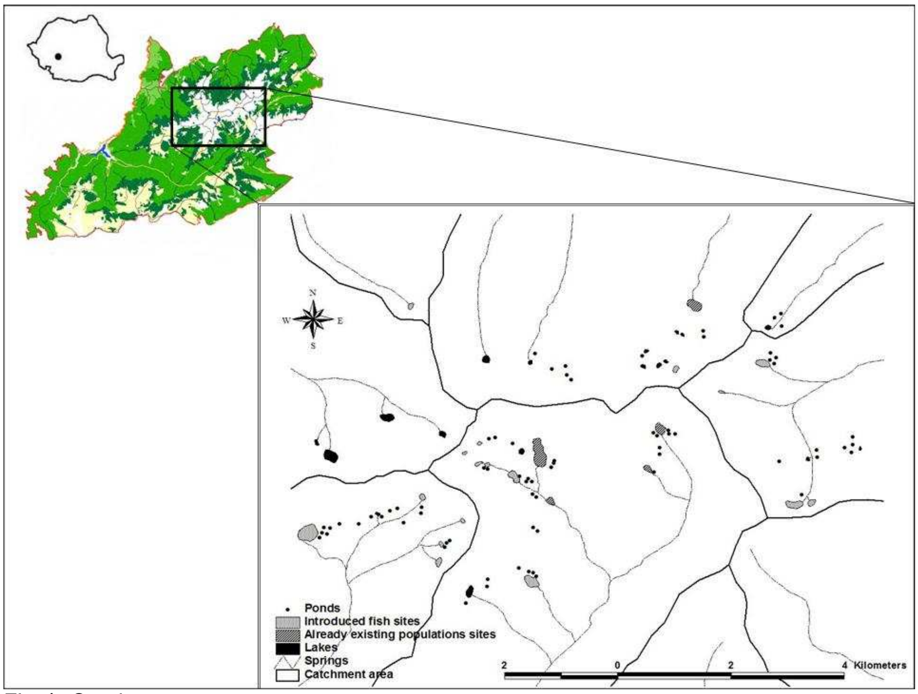

Fig.1. Study area

A total of 105 lakes and ponds were studied (Fig.6.to Fig.11). Among them was the deepest glacial lake in Romania (Zanoaga with its 29 meters depth), the largest glacial lake (Bucura with its 8.9 hectares), and one of the highest glacial lakes in the whole mountain range in Romania (Portii lake, 2230 meters altitude).

Originally all water bodies lacked salmonid fishes, but 7 lakes have been naturally populated with brown trout, *Salmo trutta fario*. The fish populations came into the alpine lakes from the rivers around the alpine area which are connected with some of the lakes through the small creeks and springs (Decei 1981). Fish introduction sessions started in 1961 and went on until 1977 when 17 lakes had been stocked with rainbow trout *Oncorhynchus mykiss*, lake trout *Salmo trutta lacustris*, brook trout *Salvelinus fontinalis*, northern whitefish *Coregonus peled*, and the already regionally present brown trout. (Decei 1981). Two of the lakes that received new fish populations already contained brown trout that had immigrated naturally, and new introduced species have naturally spread into two other lakes which already contained brown trout. In 8 lakes the brook trout was introduced, 5 lakes were stocked with rainbow trout, 4 lakes with lake trout, another 4 lakes with northern whitefish, and 9 lakes with the brown trout. Because of the repeated fish stocking sessions with different species some of the lakes were stocked with two or more species. At the end of 1977 there were 22 lakes containing different fish populations.

In some of the lakes the fish populations failed to become established due to improper physical and chemical conditions. Even in lakes where populations became established subsequential extinctions have been recorded. Some of the lakes lost their fish populations just after a short period of time following the introduction, whereas in other cases fish populations have remained for at least a number of years. The main reason for stocking was to produce more sites for recreational fishing. Fishing was very restricted immediately after the fish introductions. Now all the lakes are open for fishing and the number of licenses given has increased.

There are seven species of amphibians found in the Retezat Mountains including fire salamander *Salamandra salamandra*, common newt *Triturus vulgaris*, alpine newt *Mesotriton alpestris*, yellow-bellied toad *Bombina variegata*, common toad *Bufo bufo*, common frog *Rana temporaria* and agile frog *Rana dalmatina* (Cogalniceanu et al. 2001). Only the common frog and the alpine newt are widespread in lakes above the tree line.

The common toad and yellow-bellied toad are also present but just in few places and with low numbers of individuals (Cogalniceanu et al. 2001). The common grog is found in waters with pH ranging from 5.7 to 7.5 and the alpine newt from 5.7 to 6.3, and they occur up to a maximum altitude of 2260 meters for the common frog, and 2100 meters for the alpine newt (Cogalniceanu et al. 2001).

None of the amphibian species in the national park are listed as endangered or under threat according to the IUCN classification, but locally/regionally they may be declining

Material and methods

General survey

The field work was conducted from 10th of July to 28th of August 2006. It consisted of a general survey of lakes and ponds, a detailed survey of tadpole distribution within water bodies, and an experiment on fish predation. The general survey covered all lakes and ponds in the alpine glacial area of the National Park. All the water bodies were inventoried to see whether they contain fish or amphibians or not. The presence or absence of fish was assessed through observation from the shore and by angling. All the lakes were checked at least three times for the fish presence. Angling was mainly performed in lakes where we did not see any fishes during the shore observations. It was considered that a lake does not contain any fish when no single fish was seen or caught by angling. The available data for fish introductions in the area was taken from existing literature and from discussions with local anglers and local forest department officers. All the water bodies were categorised in three categories: fish present, fish present earlier but now extinct and fish never present.

The number of the adult amphibians in lakes and ponds of the entire study area was counted and the abundance of larval amphibians was censused by observation from the shore and by snorkel survey (Tyler et al. 1998, Denoel et al. 2005). The entire surface of the ponds and marshes was surveyed. For the big lakes a two meter strip along the entire shoreline was surveyed. A two meter strip is appropriate to estimate the number of tadpoles in the entire lake because they have the tendency to stay in shallow waters where the water temperature is higher, and they have more alternatives to hide in order to avoid predation. The purpose of this survey was to estimate the number of tadpoles in lakes with and without fish.

The vegetation in the surrounding area was also documented to see if the amphibians have any preferences in choosing places for breeding. In this way I took data about the vegetation along the shoreline of all the waters, the water and air temperature were collected and also the physical waters characteristics were noted: the area was calculated using the GPS system, the median and maximum water depth for the big lakes was taken from the literature and it was measured for the ponds and marshes. I also tried to find out if the dispersal barriers around the lakes could influence the number of places with tadpoles and in the same time to influence the number of tadpoles in all these lakes and ponds. The distance to the closest water with tadpoles was measured from GIS maps and it was noted whether there is any dispersal barrier between the present water and the closest water with tadpoles

Detailed survey of tadpoles

Three lakes with fish and eight without fish were chosen for a more complex survey. It was conducted in order to get a better estimate of the number of tadpoles and to note the difference between the tadpoles' behaviour in lakes with fish versus lakes without fish. I chose these 11 lakes from the 36 big lakes because they were the only ones to hold tadpoles of the common frog. I was also looking at the shoreline characteristics for the proportion of shallow water which is important for tadpoles.

Four 25 m segments of the shoreline were randomly chosen along the perimeter of all 11 lakes. Each segment was surveyed by walking or snorkelling along two transects parallel to the shore. I did not snorkel where the water was less than 1 meter deep because in these parts of the lakes I was able to walk and perform a normal visual survey. The first transect was two meters wide and followed the shore-line, and the second transect was placed at a mean distance of five meters from the shore, and measured three meters wide (Tyler et al. 1998).

The number of tadpoles within each transect was estimated. Approximate numbers had to be used due to the large number of tadpoles in some of the lakes. Estimations were made by counting the number of tadpoles in one square meter and multiplying this by the total area were tadpoles were observed. In each water the approximate number of tadpoles hiding under rocks or other substrate materials in order to avoid predators was estimated. Only rocks and substrate material that I considered could provide hiding places for tadpoles were searched. No rock or other substrate material for hiding was moved from its initial place after the search was completed, meaning that all the rocks and substrate materials that could provide shelter or hiding places for the tadpoles were placed in the original place after the search was completed.

Predation experiments

The predation experiments were conducted in order to find out the impact that the fish introduction has on amphibian populations. They were conducted in artificial enclosures built along the lake shoreline, stocked with fish and tadpoles, and compared to tadpole survival in control enclosures. All predation experiments were conducted in two selected lakes with fish present, Lake Bucura and Lake Ana. The experiments were with the lake trout in the first lake and brown trout in the second one. The enclosures were fenced with 2 millimetres wire mesh, at least 1.5 meters in height, which was secured to the bottom with rocks and sealed with sand and debris. The substrate was typical for the lake with sand, gravel and bedrock. The enclosure made in Lake Bucura measured approximately 9 m^2 and had a volume of approximately 5 m^3. The enclosure made in Lake Ana measured approximately 8 m^2 and had a volume of about 4 m^3. Each enclosure was divided into two equal parts. In each experiment, one part contained fish and the other part was used as a control (Gillespie 2001). In each part of the enclosure there were at least five hiding places to provide the tadpoles with refugee sites.

The two most widespread fish species were selected for the experiments, the brown trout and the lake trout. These two subspecies represent one form, the brown trout, that has been present for a long time in some of the lakes, and one that was more recently introduced. Trout fishes were caught by angling and temporarily placed in large containers to recover for at least 12 hours prior to the experiments. All fishes used were considered large enough to consume tadpoles. The length varied between 18.8 and 23.7 cm, with a median value of 21.2 cm. The difference in body length between the two fish species was small (brown trout 18.8-23.7 cm, mean=20.9 and lake trout 19.2-23.6 cm, mean=21.5 cm). The water temperature during experiments never exceeded 20ºC, the limit above which the trout may suffer heat stress (Cadwallader and Backhouse 1983). Gut samples were taken from each trout used in the experiments and from other trouts caught by anglers in the area, in order to identify the prey species. The gut content was conserved in 95% ethanol and was brought for analyses to the Biological Research Institute (ICB) from Cluj Napoca, Romania.

I used tadpoles of common frog for all the experiments. The tadpoles were caught using hand nets and placed in containers until they were released in the enclosures. The tadpoles were collected from the nearby ponds for the experiments held at Lake Bucura and from the lake and nearby ponds for the experiments at Lake Ana. All tadpoles were measured prior to the experimentation and their developmental stage was recorded (after Gosner 1960).

In each replicate a total of 40 common frog's tadpoles were added in each enclosure, with 20 in the section with one fish and the other 20 in the control part. After two days the tadpoles were removed and counted using repeated dip-net searches. Searches were repeated until no more tadpoles could be captured during two consecutive 30 minutes searches. This recovering procedure was evaluated in the pools prior to experimentation. A number of 20 tadpoles were added in one side of the enclosure before all the experiments started and were left there from afternoon until noon the next day when I started to search for the tadpoles. Repeated searches were done every 30 minutes until no more tadpoles could be located or all 20 tadpoles were found. In this way I was able to locate and catch all 20 tadpoles after three repeated searches. I did not count the remaining number of tadpoles that were hidden in the control part of the enclosure because I could not count all of them at first sight and after they were trying to hide. The counting of hidden or not hidden tadpoles was done just in the experiment part of the enclosure.

For each replicate a new trout, and a whole set of new tadpoles were used, so that each replicate started with animals of equal experience with the setup. A total of 26 experiments were done, with 13 experiments for each lake. An equal number of trials were done for each fish species.

Statistical analyses

Chi-Square tests and Fisher's exact tests in 2x2 tables were used to compare the proportion of adults and tadpoles of the common frog and the alpine newt from both categories of lakes. Fisher exact test was used to compare the proportion of newts in the ponds with and without dwarf pine in the near surrounding area. Another nonparametric test – Mann-Whitney U test - was also used for comparisons between groups. Analysis of variance was used to find if the fish length had any influence in the predation level. The fish size was included as a covariate. ANOVA with interaction terms was also used for the experiments part to find out which independent variable or a combination of independent variables affects the value of the dependent variable. The dependent variable used was the number of tadpoles that survived after each experiment.

Results

General survey

During the general survey 105 suitable waters for amphibian reproduction were identified, out of which 69 were ponds or marshes and 36 were small, medium or big lakes. I considered being pond all the water with the maximum depth less than one meter. All the other lakes with maximum depth over one meter were considered to be lakes.

The surveyed waters ranged in size from 0.002 to 8.9 hectares with a median value of 0.52 hectares, with an estimated maximum depth from 0.15 to more than 8 meters (with a median value of 2.09 meters).

The rainbow trout, the lake trout, brook trout, northern whitefish or the brown trout were found in 12 of the 22 lakes initially stocked (Fig…). Some of the lakes were containing just brown trout, and the rest of them had a mixture between the new introduced species and the brown trout. This means that 10 lakes that previously supported fish populations now had lost them, and that 83 waters were considered never to have received any fish.

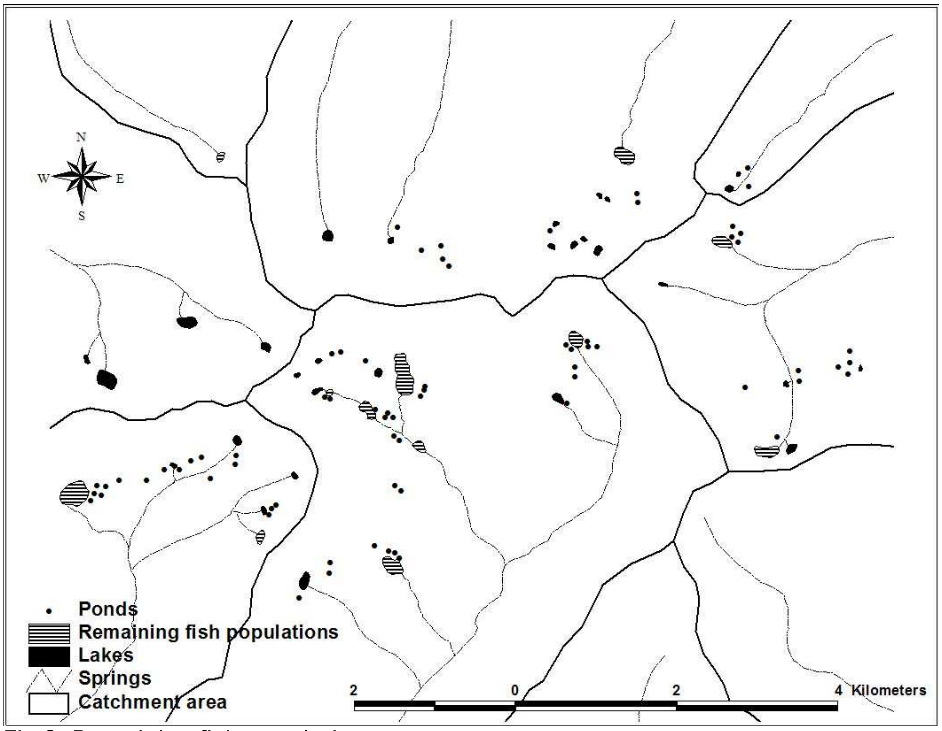

Fig.2. Remaining fish populations

Among the 36 big lakes, the lakes with fish were larger in area than the lakes without fish, with a median surface of 2.9 ha in the lakes with fish and 0.52 ha for the lakes without fish (Mann-Whitney U-test, U=20.5, z=4.14, p=0.0034) and also deeper than the lakes without fish with a median depth of 9.7 m, as against 2.25 m in the fishless lakes (Mann-Whitney U-test, U=12.5, z=4,26, p=0.0022). There was also a significant difference between the area of the lakes that still have fish populations and the lakes that used to have fish (Mann-Whitney U-test, U=9.5, z=3.3, p=0.0008) and also between the maximum depth of the two lake categories (Mann-Whitney U-test, U=14, z=3.03, p=0.002).

The amphibian species found in the area were adults, juveniles and tadpoles of the common frog and adults of the alpine newt (Table 1). They were found in 69 lakes and ponds out of the total of 105 waters. Adults and juveniles of the common frog were found in 38 lakes and ponds, among which 8 also contained fish populations. Tadpoles of common frog were found in 38 lakes and ponds, and three of them also contained fish populations.

Table 1. The number of waters containing tadpoles of the common frog and adults of the common frog and of the alpine newt.

	Tadpoles	Ad./juv. frogs	Newts	Total number of waters
Lakes with fish	3	8	0	12
Previously with fish	2	2	1	10
Always without fish	33	28	28	83

Water depth effects
All six amphibian parameters respectively frog adults, both males and females, frog juveniles, frog tadpoles and also newt adults, males and females were tested to see if there are any relations between their numbers and median and maximum water depth. I found that the number of adults of common frog were related to the maximum water depth (Anova-GLM, F=1.77, d.f.=27, p=0.027 for males and F=2.33, d.f.=27, p=0.002 for females). No relation between the number of adults frogs both males and females and the median water depth was found. All the other four amphibian parameters were not related to the median and maximum water depth (Anova-GLM tests). All there tests were performed just for 69 lakes and ponds where at least one of the amphibian parameters were found.

Fish effects
The distribution of frog adults in all 105 waters seems positively affected by the presence of fish, as they were found in 8 of 12 lakes with fish, and in 32 of 93 lakes and ponds without fish (Fisher exact test, p=0.054).The distribution of tadpoles was not related to the distribution of fish. They were found in 3 of 12 lakes with fish and in 35 of 93 lakes and ponds without fish (Fisher exact test, p=0.53). The tadpoles in the three lakes containing fish were found in the very shallow waters, hiding among the stones. Alpine newt adults were not observed in any of the 12 lakes which still have fish populations but were found in 29 lakes and ponds without fish (Fisher exact test, p=0.03). Moreover, the alpine newts showed an affinity with ponds surrounded by the dwarf pine *Pinus mugo,* being found in 24 ponds with dwarf pine out of a total of 46 ponds which were surrounded by the pine, and in just 5 ponds without pine (Fisher exact test, p= 0.0). No alpine newt larvae were observed during the field work. Taking into consideration just the 69 lakes and ponds where amphibians were found the number of tadpoles of common frog was related to the sites that contained fish in the past but now went extinct (Anova-GLM, F=4.3, d.f.=1, p=0.046). None of the six amphibian parameter numbers were found to be related to the actual fish distribution (Anova-GLM tests).
The number of tadpoles of common frog were highly related to the surface area (Anova-GLM, F=3.65, d.f.=13, p=0.002). The number of adult newts

were also found to be related to the distance to sites with frog tadpoles (Anova-GLM, F=2.11, d.f.=16, p=0.037).

The ridges and precipices seem to be a very important factor in the distribution of amphibians in the alpine area. Tadpoles of the common frog were found in 28 of the lakes and ponds from a total of 53 which were not separated by such dispersal barriers from the nearest water with amphibians present, and in just 10 lakes and ponds out of 52 affected by dispersal barriers (Chi square test on presence/absence of tadpoles in ponds with and without dispersal barriers, Chi-Sq = 8.225; DF = 1; p= 0.004).

Detailed survey of tadpole distribution

The surface of the 11 lakes taken for further studies ranged from 0.1 to 3.6 hectares with a median value of 1.72 hectares, and maximum depth ranged from 1 to 29 meters (median value of 5.3 meters).

The results from the two transects (at 2 and 5 m away from the shoreline), (Table 2) along the four 25 m segments of each lake show a great difference between the number of tadpoles found at 2 respectively at 5 meters from the shore in the two types of lakes. Out of the total number of tadpoles found in the lakes with fish, just 4.8% were found in the 5 m transect. The remaining 95.2% were found in the 2 m transect in very shallow waters, most of them trying to find a place to hide. In lakes without fish 85.6% of the tadpoles were found in the 2 m transect and 14.4% in the 5 m transect.

Table 2. Average number of tadpoles and average density per square meter.

	Fish 2m	Fish 5m	No fish 2m	No fish 5m
Average number	1333	50	1850	312.5
S.D. in number	1025	50	1347	164.2
Average density/sq.m.	0.7	0.03	2.94	0.49

The transect position had a very strong effect on the number of tadpoles (F=10.42, d.f.=1, p=0.005), but there was no significant effect from the fish presence (F=0.79, d.f.=1, p=0.38) and also no effect from the interaction term between fish presence/absence and the transect position (F=0.08, d.f.=1, p=0.77).

Predation experiments

No trout escaped from the experimental enclosures, so all 26 replicates were taken for further analysis. Both fish species combined had a very strong effect on the number of tadpoles that survived the predation experiments (F=1031.95, d.f.=1, p=0) (Fig 3),

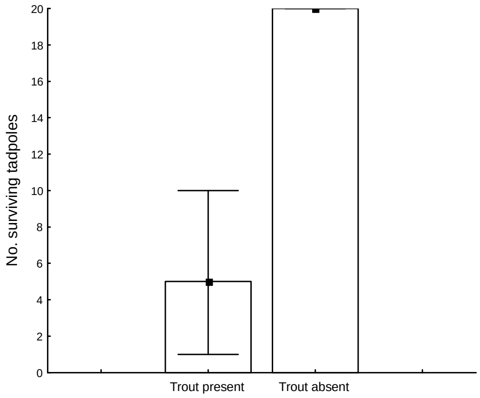

Fig.3. Mean and standard errors of numbers of tadpoles that survived in the presence and absence of trout.

but there was no difference between the two fish species in their predation level on tadpoles. The brown trout species had eaten 71.9% of the total number of tadpoles used in the experiments, and the lake trout species had eaten 77.6% of the tadpoles (Mann-Whitney U-test, U=59.5, z=1.28, p=0.19) (Fig 4).

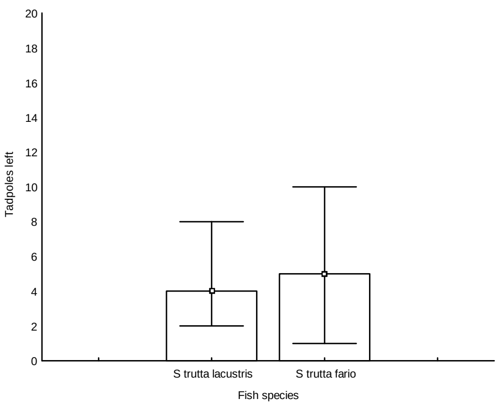

Fig.4. Mean and standard errors of numbers of tadpoles that survived in the presence of Salmo trutta lacustris and Salmo trutta fario during predation experiments.

There was no difference between the two sections of each enclosure regarding the number of tadpoles eaten in each experiment (F=1.45, d.f.=3, p=0.2), and there was no effect on the predation level from the interaction between the two subspecies of fish used in the experiments and the two sections of the enclosure (F=1.458, d.f.=3, p=0.23).
The fish size did not significantly affect the predation levels (F=1.72, d.f.=5, p=0.17). All the Anova results for the predation experiments are deriving from

one Anova GLM test with fish species, section of the enclosure, size and also the interaction between fish species and section of the enclosure included as independent variables and the number of tadpoles as dependent variable. There was also no difference in the predation level on the tadpoles between the two lakes used for the experiments (Mann-Whitney U test, U=313, p=0.62). Among the surviving tadpoles 75.5% in the experiment part of the enclosures were found under loose rocks. In the experiments with brown trout 69.8% of the remaining tadpoles were found hidden under rocks and also 82.75% of the tadpoles were hidden in the lake trout experiments. There was no significant difference between the two different fish species in their effect on tadpole hiding behaviour (Mann-Whitney U-test, U=64.5, z=1.02, p=0.30) (Fig 5).

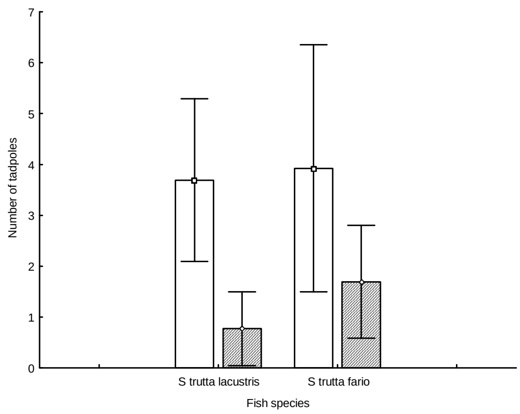

Fig.5. Mean and standard errors of numbers of tadpoles that survived (open bars - hidden tadpoles and solid bars - not hidden tadpoles) in the presence of the two fish species.

The stomach content analyses have revealed other food items but not tadpoles. The results have showed that the most commonly eaten preys apart from tadpoles were trichoptera larvae, amphipod larvae and many flies.

Discussion

Surveys

The results of the study suggest that the introduction of fish has had a very strong negative effect on the aquatic fauna. The adults of the common frog, being found in low densities in some of the lakes containing fish, seemed to be less affected by the presence of the predatory fish. The tadpoles of the common frog were abundant in two of the lakes stocked with fish and very few or absent in the other lakes stocked with fish. The adults of the alpine

newt were totally absent from the lakes with fish. The size of the prey is without doubt a limiting factor in the predation process. The fish must be big enough to be able to consume juvenile and adult frogs. Yet, in some of the lakes there are big fishes capable of eating even adults. The biggest trout caught during the field work measured over 65 cm in length.
The adults of the common frog were related to the water depth which means that they need deeper waters. They were not laying eggs in some of the big lakes but they were using these lakes or the shoreline around these lakes later on most probably because they could find more food. And probably some of the adults are overvintering in water which should be deep enough not to be affected by the frost or by lack of oxygen.

There is an ongoing discussion on the relative importance of the present and historic fish distribution patterns for the status of amphibian populations. The fact that fish still exist in some of the lakes is a good indicator that those lakes will not be recolonized by amphibians as long as the fish populations are present. There are no studies that show the distribution of frogs and newts before 1961, when just 7 of the lakes in the alpine area contained fish. The fish impact on amphibians might have been stronger when some more lakes have been populated with fish and during the period that followed the fish introduction. This view is supported by the fact that some of the lakes stocked with fish have the best conditions to sustain amphibian populations, such as shallow water at the shore, and vegetation around the lake which provides adults with food, and yet they now lack amphibians. These lakes were smaller that the lakes that still hold fish populations and usually these lakes do not have many hiding places available where the tadpoles can hide. In his way the predation rate may become higher than in the big lakes (Denoel et al. 2005).
The fish disappearance process may have been caused by one or several different reasons. The scarcity of food after a period following the introduction might be one of the reasons: once the fish had exterminated the amphibians the food became scarce and maybe not enough to maintain the existing fish population, causing the subsequent extinction of the fish as well. Problems with oxygen shortage during overwintering may also cause fish extinctions. This lack of oxygen might have as a cause the long winter period when the lakes are ice covered, sometimes even five months.

The common frog is found in many different places, from very small ponds to big lakes, both with fish and without fish. The alpine newt seems to be quite selective in its choice of habitats: is never found in lakes stocked with fish, and also it has a preference for the waters surrounded by dwarf pine. The alpine newt seems to be more susceptible to fish predation. This difference in apparent susceptibility to fish predation may have different causes. The differences in life traits between the two species may contribute to the differences in susceptibility. The number of eggs laid by a female of alpine newt is much lower than what a common frog can produce. Another factor may be the spawning process which is also very different in the two species; the females of the common frog lay the eggs freely in the water, whereas the females of the alpine newt need vegetation as a substrate for their eggs. There are also differences in larval feeding behaviour: the common frog tadpoles are feeding at the bottom of the lakes or ponds and the alpine newt tadpoles are feeding in open waters. In a harsh environment with limited food availability and a short period with good weather conditions during the summer, the percentage of surviving tadpoles from both species may be very low even without introduced predators.

The big difference between the numbers of tadpoles in the two transects in both fish-inhabited and fishless lakes demonstrate that there is a disturbing factor which leads to this difference in numbers. And the disturbing factor is the predatory fishes which change the behaviour and movement of tadpoles in the way that they avoid places with deep water where they would be more exposed to predation. Trout may not directly eliminate the two amphibian species but restrict populations to optimal parts of the lakes or even to optimal lakes and ponds in the area having as a direct effect the fragmentation of the existing populations. That would in turn increase the vulnerability to local extinction (Gillespie 2001). Some of the populations may not disappear immediately following the fish introduction. They may persist in low numbers for years but this process depends on factors such as habitat characteristics, disturbance regimes and seasonal factors (Gillespie 2001). Sometimes the only available fishless places were temporary ponds which part of them are subject to depletion during the summer and the water in them do not last enough to allow tadpoles to reach metamorphosis. Furthermore, once that the isolation has occurred, the probability of recolonization of the sites where extinction occurred is much reduced, compared to other more calm environments, even though the fish may have disappeared (Bradford et al. 1993). This is even more true in the alpine environment, in which the dispersal barriers are an important factor in the recolonization process following local extinctions. When the amphibians disappear from one place, a population may reappear through recolonization. The results of this study have shown that there are fewer waters with tadpoles in areas isolated by dispersal barriers from other waters with tadpoles present.

All tadpoles found could be assigned to one of two distinct age groups with different traits. The older ones were trying to hide when someone was around the lake or the pond. The younger ones were most of the time found in big groups and with no obvious hiding tendency. A direct consequence of this observation might be a higher predation level on the younger stages of tadpoles. Even though the place where the tadpoles were found might not be reachable by the big fishes, it is mostly reachable by the smaller fishes. All the tadpoles used in the experiments belonged to the first category described above.

An unexpected factor in this big equation was the presence of Eurasian Minnow *Phoxinus phoxinus* which may influence the predation level on tadpoles in the lakes where it was introduced. The anglers and the poachers have introduced *Phoxinus phoxinus* as a minnow for the trout. The number of lakes where this little fish exists is not known. It has probably increased over several years. I assume that the large number of tadpoles in two of the lakes stocked with fish is due to the Eurasian minnow presence as a better food option for fishes.

Experiments

I tried to design the experiments in such a way that would keep as much as possible of the natural conditions in the lakes. My opinion was that using containers or other small recipients for experiments would have not shown the real ability of trout to prey on tadpoles and it would have not shown the real impact into a real lake. The difference between experiments performed in containers or other recipients and experiments in small enclosures separated from the lakes is that the trout in enclosures have access to other types of food, and the tadpoles have the possibility to seek refuge and hide under rocks and gravel on the bottom of the lakes. For this purpose I constructed at least five hiding places in each enclosure where the tadpoles could hide. This could cause the predation level to be lower than in more unnatural experiments. Still the trout in this experiment showed the capacity to severely affect tadpole populations.

During the experiments both fish species have drastically reduced the survival of tadpoles of common frog even though they had hiding places, some of them being inaccessible to trout, where the tadpoles could find refuge. The results of the experiments have also shown that there was no difference in the predation level between the brown trout and the more recently introduced lake trout, which means that the tadpoles are palatable for both species to almost the same extent. I was expecting to find that the longer-established fish species would have a lower impact since the tadpoles may already have developed an adequate strategy to avoid their predation. But it seems that even if they have already developed some sort of survival strategy, e.g. by trying to leave the waters with fish and breed in adjacent waters that would not be very effective in the direct predation experiments.

The fact that such a large percentage (75.5%) of the total surviving tadpoles in the experiments stopped was found under loose rocks mean that they were trying to hide in order to avoid predation by the trout.

The laboratory results based on the stomach content samples have shown that other aquatic taxa are also under pressure in the lakes where the fish species are present. We failed to identify any tadpoles in the fish stomachs. We expected this result considering that the tadpoles are soft body organisms and that they would be digested very quickly. Other food items in the stomachs included flies, a large number of trichoptera larvae and amphipod larvae (Crustacea).

There are a number of limitations to the experimental design. The enclosures were artificially constructed. Maybe the predation rate would have been lower into a natural pond. The fact that part of the tadpoles used for experiments were taken from nearby ponds might have influence the predation level even though there was no big difference in the predation level during all trials. I was thinking here that the tadpoles from the lakes with fish are under different stress factors than the tadpoles from the nearby ponds which can lead to a different behaviour during the experiments.

Another limitation might be on the availability of other prey items in the experiment enclosures which made the predation level on tadpoles even higher. However, I did find alternative food items in the stomach samples.

The results of the study show that, despite the fact that the tadpoles live mostly in very shallow waters where the trout basically does not have access, the tadpoles are still vulnerable to predation from fish. It would have been very good to try to include also Eurasian minnow in the predation experiments and try to see which of the both, tadpoles or Eurasian minnow is mostly preferred by the trout. In this way I might have got an answer why are there so many tadpoles in the two of the lakes stocked with fish which also contain many Eurasian minnow.

Another important aspect on the predation level may be the impact that fingerling trout with the body size up to 10 cm in length have on earlier amphibian tadpoles' life stages because they have higher accessibility to shallow shorelines. It would have been interesting to perform experiments with fingerling trout too to see if there are any differences in their predation level.

The introduction of fish species in these alpine lakes has drastically reduced the number of individuals from other species as well. The brachiopod species *Chirocephalus diaphanous* has decreased in numbers of individuals and of the places where it occurs, since the introduction of the fishes (Demeter & Mori 2004).

A four year study conducted on the river valleys across the National Park's borders has revealed that the human being is responsible for the killing of thousands of adult frogs in early spring during the mating season (Dragos

Cocos, unpublished data). They are killed for meat consumption. This phenomenon was not reported in the alpine area. It means that the populations all over the National Park are under big pressure. There is a lack of places for amphibian breeding between the alpine area and the river valleys and sometime the adults from mid-high lands are going to breed in certain ponds in the river banks, where many of them are killed by humans. Even though the Park rangers are often patrolling along the valleys in the breeding season, the mortality rate among the adults is high every year.

Conclusions and recommendations

The introduction of fish species can lead to local extinctions among the amphibian populations either through direct predation or population fragmentation which may cause local extinctions in these harsh environments. The results of this study have shown that the fish introduction in the alpine lakes had a negative effect on amphibians. Other studies have shown that the fish introduction had a negative effect on other aquatic communities as well (Demeter & Mori 2004). Restoration efforts should also be taken into consideration by removing the introduced fish in the lakes, if at all possible. Long term monitoring programs should be set up in order to help the recovery process for the two amphibian species taken for study. The recovery process should be a natural one in the places where there are still viable populations and where the area is suitable for recolonization by its own and this is why a recovery process monitoring is needed.

Legislation on stopping the nonnative fish introduction at national level should be also considered.

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Appendix I Photoseries of studies site lakes

Fig.6. Peleguta lake

Fig.7. Galesu lake

Fig.8. Taul Tapului lake

Fig.9. Pietrele lake

Fig.10. Valea Rea lakes

Fig.11. Lia lake

Fig.12. Rana temporaria juvenile