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THE IMPACT OF FISH ON AMPHIBIAN METAMORPHOSIS A STUDY CASE FROM RETEZAT NATIONAL PARK, ROMANIA

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Abstract. *Fish introductions are a threat to freshwater biodiversity, particularly in historically fishless alpine lakes. Amphibians, already in global decline, are highly susceptible to disturbances during their aquatic developmental stages. In Romania, the widespread fish stocking with trout during the 1960s of alpine lakes has resulted in the establishment of the common minnow (Phoxinus phoxinus)—a bait species—presently co-occurring with native amphibians. We conducted a mesocosm experiment to assess the impact of minnows during the early developmental stages of common frogs. We quantified: (i) survival to the onset of metamorphosis, and (ii) endurance, in freshly-metamorphosed froglets. While survival to metamorphosis was significantly lower in the presence of fish, no significant differences were detected in size, body condition, or endurance of metamorphs. The absence of observable carry-over effects suggests possible early-stage compensatory responses, though subtle sublethal effects may have been missed. These findings highlight the importance of conserving fish-free alpine habitats and raise concerns about the overlooked impact of bait fish on amphibian recruitment.*

Keywords: Carpathian Mountains, Phoxinus phoxinus, bait fish, Rana temporaria, protected area.

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Introduction

Alpine ecosystems are biodiversity hotspots facing considerable threats from fish introductions worldwide [38], driven by human activities like sport fishing [37,49]. Fish stocking poses a particularly significant threat to historically fishless environments, since fish disrupt trophic structures and ecological processes and interactions, leading to negative cascading ecological effects [24,6].

Such disruptions have long-term ecological consequences for various organism groups and ecosystem functionality [24,2] even after fish removal [31,47]. Conservation evidence emphasizes the need for fish eradication to restore ecosystem functioning and stability [37].

Introduced fish pose severe threats to amphibian populations through multiple mechanisms, including predation, competition for resources, habitat modification, disease transmission, and broader, indirect effects on the ecosystems [49,24]. There is strong evidence showing that fish introduced in alpine lakes and streams disrupt amphibian behavior and habitat usage, leading to population declines [44,48,31]. Amphibians tend to avoid fish-dominated habitats due to heightened predation risks, resulting in reduced breeding success and habitat fragmentation [6,27]. These disruptions extend beyond immediate predation and competition, triggering cascading ecological effects, such as shifts in vegetation cover and water quality, that in turn compromise amphibian breeding sites and larval development success [20,27,53]. Additionally, climate change exacerbates these threats by altering hydroperiods and temperature regimes, thus decreasing amphibian survival rates [21, 10,3].

Another critical issue is related to the spread of baitfish through recreational fishing, particularly minnows (*Phoxinus* sp.), which disrupt alpine ecosystems through predation on benthic macroinvertebrates, and zooplankton [25,36,13]. Unlike salmonids, minnows have a higher acclimation success and invasive potential [7], and are more likely to survive at higher temperatures. Minnows tend to become the dominant fish species and reach high densities in alpine lakes where they are introduced, particularly where trout populations have declined [45]. Their small size allows them to access the shallow areas inaccessible to trout, interacting with amphibians that use the littoral areas as a refuge [25]. Their presence was linked to significant reduction of reproductive success in amphibians, particularly in species like the common frog (*Rana temporaria*) [29]. Common frog populations inhabiting alpine habitats (i.e., at the limits of their altitudinal range) are particularly vulnerable to habitat degradation, climate change, and introduced fish, which significantly reduce breeding success and alter habitat-use patterns [24,30].

Environmental stressors such as predation risk, resource availability, and hydroperiod length influence larval growth rates in amphibians, which can result in reduced energy reserves, lower body size and diminished endurance at metamorphosis and post-metamorphosis [41,43]. Larger size at metamorphosis is typically associated with enhanced locomotor performance and greater endurance, allowing individuals to better evade predators and secure food resources in the terrestrial stage (e.g., Vonesh 2005 [50]). Amphibian larvae exposed to environmental stress may show carry-over effects in later developmental stages, like reduced jumping ability, lower aerobic capacity, and therefore increased vulnerability to terrestrial predators [40]. These findings emphasize the role of habitat quality during larval growth in shaping fitness trade-offs that extend into later life stages.

A two decade-long fish stocking program initiated in Romania in the 1960s introduced both native and non-native trout species in alpine lakes in Retezat National Park (RNP) [11]. The park has 58 permanent glacial lakes and a similar number of temporary lakes, distributed between 1700 and 2300 m a.s.l. [32]. The stocking program targeted the naturally fishless alpine lakes from RNP, which are used as breeding sites by the local amphibian communities (i.e., common frogs, alpine newts and common toads). While only a few self-sustaining trout populations persist today, the Eurasian minnow (*Phoxinus phoxinus*), which was introduced as bait, thrived. Currently, amphibians and minnows co-occur in alpine lakes within RNP, which provides an ideal setting to study species interactions.

We conducted a mesocosm experiment to assess if the presence of minnows has an impact on the early development of common frogs. More specifically, we quantified: (i) survival to the onset of metamorphosis, and (ii) endurance, in freshly-metamorphosed froglets.

Material and methods

Study Species. The common frog is one of the most widespread amphibians in Europe, classified as Least Concern due to its broad distribution and stable populations [55]. This species displays high environmental plasticity, enabling it to thrive in diverse climatic conditions, from lowland forests to high-altitude alpine meadows above 2000 m [33,8]. During colder months, *R. temporaria* undergoes hibernation, typically in muddy pond bottoms, beneath logs, or buried in soil, re-emerging with rising temperatures in early spring [15]. Breeding occurs in temporary or permanent water bodies, where females lay large clutches of eggs in communal spawning sites to enhance offspring survival.

The developmental rate of tadpoles is highly influenced by temperature, hydroperiod length, and predation risk, with high-altitude populations often exhibiting prolonged larval periods due to colder water temperatures [14]. In Romania, *R. temporaria* is one of the few amphibians that occurs in alpine ecosystems [9].

The Eurasian minnow (*P. phoxinus*) is a small cyprinid fish native to lowland and subalpine freshwater systems across Eurasia. It was widely introduced into mountain lakes, where it was historically absent [24,45]. It thrives in cool, well-oxygenated streams, rivers, and lakes, often coexisting with the brown trout (*Salmo trutta*) in lower-altitude waters [26]. Minnows are omnivorous and opportunistic foragers; they primarily consume zooplankton, benthic invertebrates, and fish larvae [18]. Their high reproductive rate, gregarious nature, and adaptability allow them to thrive in high-altitude environments, outcompeting native species and causing significant ecological shifts [4].

Sampling and experimental design. In June 2023, we collected 60 similarly sized minnows from a lower-altitude site (Ostrovel village, N 45.5058, E 22.8477, 460 m, Fig. 1). We selected this location because of its accessibility and ease of transporting the fish to the location where the experiment took place.

We collected 10 fresh common frog egg clutches from Bucurelu lake, a fishless temporary lake at 2070 m in RNP (N 45.360501, E 22.871043) (Fig. 1).

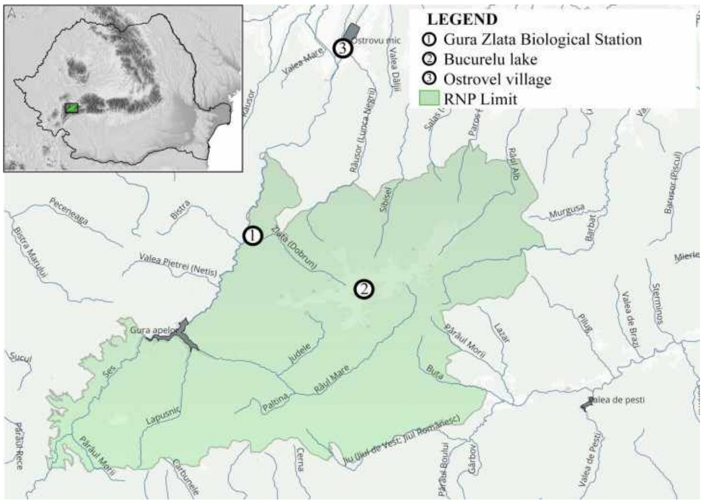

Fig. 1. Location of Retezat National Park (inset image - position in the Romanian Carpathians), the site where the experiment was conducted (1. Gura Zlata Biological Station), and the two sampling sites for the common frog egg masses (2. Bucurelu) and fish (3. Ostrovel)

We conducted the experiment at Gura Zlata Research Station, Retezat Mountains, Romania (N 45.391504, E 22.773070, 800 m, Fig. 1). The experimental setup was a mesocosm with a control (no fish, n=8 replicates) and a fish-presence group (n=8 replicates), distributed across 16 plastic 500 L tanks (Fig. 2). Thus, each experimental group included eight tanks, each tank being considered as a replicate. The tanks were lined with 2 cm of forest leaf litter and filled with 400 L of river water, two weeks prior to the start of the experiment. Supplemental tanks were prepared similarly, to accommodate the fish and egg clutches prior to introducing them into the experiment. The fish and egg clutches were placed in separate tanks, to allow for acclimation and hatching. The experiment started on the 1st of July 2023, when most of the common frog larvae reached Gosner stage 25-26. We selected 40 small but similarly sized minnows (average length ± SD = 50.01±0.53 mm, min-max = 42.33-56.85 mm), and introduced five in each of the eight tanks corresponding to the fish-presence group. We opted for smaller-sized fish in order to avoid the potential predation of tadpoles. We selected 560 healthy tadpoles (i.e., without visible wounds or abnormal swimming behaviour) in Gosner stage 25-26 (i.e., free-swimming, mouth development complete), and randomly distributed them across the 16 replicates (i.e., 35 tadpoles per tank) (Fig. 2). The basins were left uncovered, and food was not artificially added throughout the experiment. Water parameters (i.e., temperature, conductivity and dissolved oxygen) were measured twice - at the beginning (day 1) and towards the end (day 16) of the experiment, using a portable multiparameter tester (Hanna HI98129) and a Dissolved Oxygen Meter (Oakton DO 450). We conducted a census of all tadpoles and metamorphs on the 20th day of the experiment, to assess survival and Gosner stages.

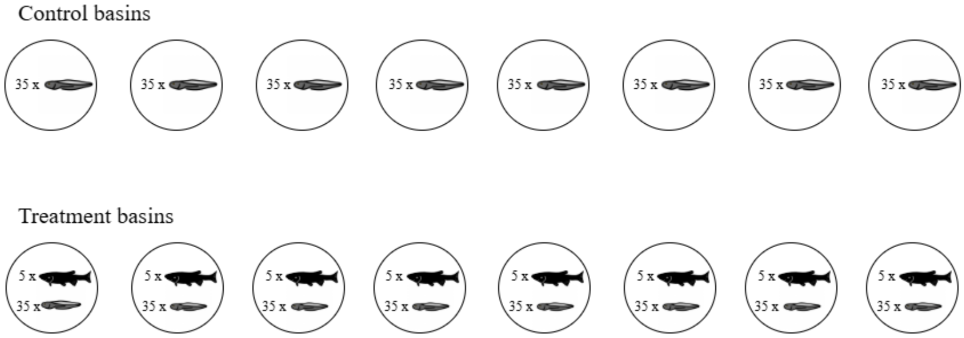

Fig. 2. Experimental design used to raise the tadpoles.

When the tadpoles approached metamorphosis, we provided them with floating supports (i.e., foam plates covered in wet moss). A subset of 28 freshly metamorphosed individuals (Gosner stages 45-46; control: n=12 individuals; fish-presence group: n=16 individuals) were weighed, measured, and tested in an arena setting. Body mass (BM) was measured using a Pesola Touchscreen Digital Pocket scale with a precision of 0.01g. We photographed the metamorphs in Petri dishes, against millimeter paper, and later measured the snout-vent length (SVL) using ImageJ v. 1.54p software [34].

We used an adapted protocol following previous studies [54,40,43], to assess metamorphs' endurance. The tests lasted 3 minutes and were conducted in a 100-cm circular arena lined with moist substrate (i.e., wet carpet) to minimize dehydration stress (Fig. 3). A metamorph was placed in the center and allowed to acclimate under a paper cup, for one minute. Then, the cup was removed. We used a wooden stick with a blunt and soft tip to gently touch the urostyle, to elicit movement, only when a metamorph remained immobile for 10 seconds. The test ended after 3 minutes or when the metamorph became unresponsive after 10 seconds, and despite receiving 3-4 consecutive taps applied at 2 second-intervals.

We recorded the tests with a web camera (Logitech HD Pro C92) placed above the arena, at a resolution of 2 Mpx and 30 fps.

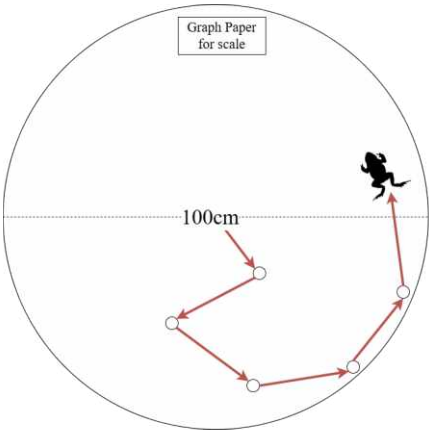

Fig. 3. Setup used for the endurance tests. The red track represents the movement of a metamorph during 3 minutes in the arena.

Data analysis. We quantified survival to the onset of metamorphosis (S_{G42}) as the percentage of individuals that reached at least Gosner stage 42, on the 20th day of the experiment. We also assessed overall survival ($S_{overall}$) on the 20th day of the experiment, as the percentage of live individuals, regardless of their developmental stage. We computed the residual body condition index (BCI) as a proxy for fitness, based on the linear regression between SVL and BM [1].

We extracted one image at each 60 frames (or every 2s) using the “scene filter” function in VLC media player software (https://www.videolan.org/vlc/), resulting in 90 frames (jpeg files) from a 3-minute video. We used ImageJ v.1.54p with the “MTrackJ” plugin [23] to analyze the activity of each metamorph during the endurance test. We computed the following parameters: 1. total distance traveled (Distance), 2. average leap length (Leap; i.e., the distance between two consecutive points of the track), and 3. time spent inactive (Inactivity, i.e., percent of instances when Leap=0). All statistical analyses were performed using SPSS v.26.0 (IBM Statistics). We chose the statistical tests according to the data distribution. Statistical significance was assessed at $\alpha = 0.05$.

Results

Water parameters were similar in both experimental groups across the experiment (General linear model, interaction effect of group and measurement date: conductivity, $F_{(1,25)} = 0.982$, p=0.331; dissolved oxygen, $F_{(1,28)} = 0.028$, p=0.869; temperature, $F_{(1,28)} = 1.090$, p=0.305).

Both survival to the onset of metamorphosis (i.e., G42) and overall survival (i.e., all stages) were higher in the control tanks (S_{G42}=55%, $S_{overall}$=86%)

compared to the fish-presence group (S_{G42}=31%, $S_{overall}$=43%), on day 20 of the experiment (Fig. 4). However, only the overall survival was significantly higher in the control (Mann-Whitney, *U*=17.000, p=0.130).

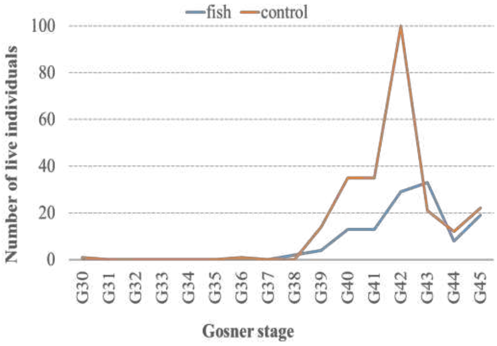

Fig. 4. Census data obtained on the 20th day of the experiment, showing the number of live Common frog individuals and their development stages.

One-way Anova tests found no significant differences between metamorphs from the control and the fish-presence group in terms of body mass (ANOVA, $F_{(1,26)}$ =2.433, p=0.131), snout-vent length (ANOVA, $F_{(1,26)}$ =0.610, p=0.442), and body condition index (ANOVA, $F_{(1,26)}$ =2.888, p=0.101) (Fig. 5).

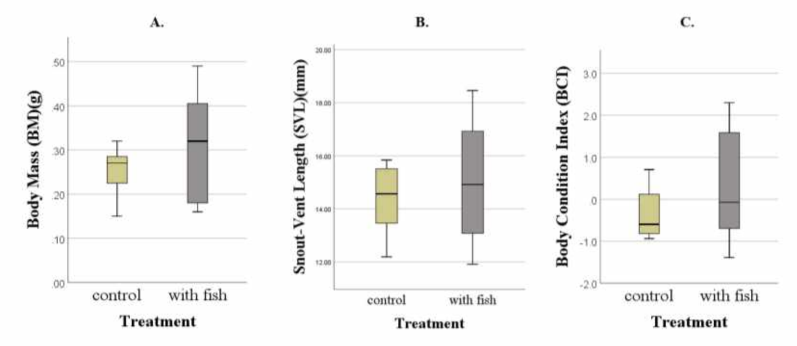

Fig. 5. Comparison between the body mass (A), snout-vent length (B) and body condition index (C) of common frog metamorphs raised in the presence (with fish) or absence of minnows (control); the whiskers represent the 95% CIs.

The Mann-Whitney U tests revealed no significant differences between the fish-presence and the control group in terms of total distance traveled (U = 94.000, Z = -0.093, p = 0.926) and leap length (U = 93, Z = -0.139, p = 0.889). Similarly, a one-way ANOVA revealed no significant difference in the time spent inactive, between the two groups ($F_{(1,26)}$ = 0.156, p = 0.696) (Fig. 6).

The descriptive statistics of the size and endurance parameters are centralized in Table 1.

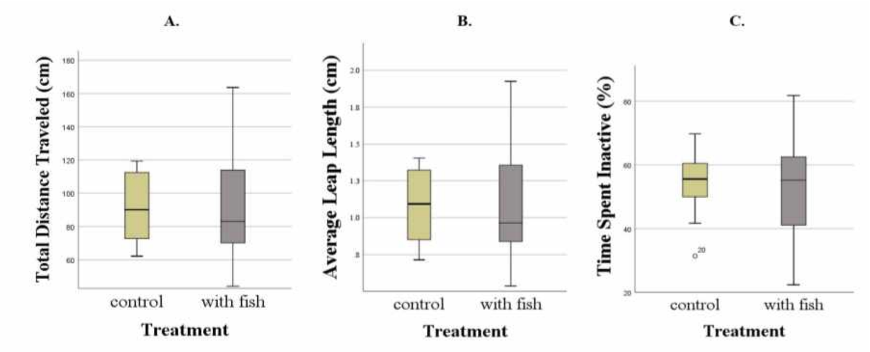

Fig. 6. Total distance traveled (A), average leap length (B) and time spent inactive (C) in metamorphosed common frogs raised in the presence (with fish) or absence of minnows (control); the whiskers represent the 95% CIs.

Table 1. Descriptive statistics of the size (i.e, SVL and BM), body condition index (BCI) and endurance parameters (i.e, Distance, Leap, Inactivity) at metamorphosis in common frogs raised in the absence (control) vs. presence of common minnows (fish). All values are expressed as average ∓ SD and min-max range. n = sample size

Treatment	n	BM (g)	SVL (mm)	BCI	Distance (cm)	Leap (cm)	Inactivity (%)
Control	12	0.25 ± 0.04(0.15–0.32)	14.4 ± 1.3(12.2–15.8)	-0.36 ± 0.56(-0.93–0.70)	97.4 ± 35.3(62.1–190.7)	1.15 ± 0.41(0.71–2.21)	54.2 ± 10.2(31.4–69.8)
Fish	16	0.31 ± 0.11(0.16–0.49)	14.9 ± 2.0(11.9–18.5)	0.29 ± 1.25(-1.38–2.30)	111.4 ± 73.3(44.0–307.9)	1.29 ± 0.81(0.53–3.46)	52.1 ± 16.3(22.4–81.8)

Discussion

Our study showed that the presence of common minnows did not significantly affect the endurance of common frog metamorphs. While survival was reduced by half in the presence of minnows, we cannot exclude the possibility that tadpoles in these tanks metamorphosed more rapidly and escaped, since our mesocosm tanks were left uncovered and only checked once a week, throughout the experiment. Prior research indicates that amphibians can adjust their developmental rates in response to predation risk [28,35]. When faced with the threat of predation, larval amphibians often alter the timing of metamorphosis, either accelerating it to escape high-risk aquatic environments or delaying it under poor growth conditions [56]. We cannot dismiss the possibility that tadpoles in our study adapted similarly, to reduce the time spent in an unfavourable environment.

Body size and fitness at metamorphosis were similar in the control and fish-presence group in our study, suggesting that minnows did not impair earlier somatic growth in common frog tadpoles. Similarly, Searcy et al. (2015) [39] found that metamorphs from fish-inhabited environments showed comparable body condition to those from fish-free habitats, indicating that non-predatory fish may not pose a major developmental challenge for tadpoles. Winandy and Denoël (2013) [52] reported that non-predatory fish could influence amphibian behavior, but their presence did not lead to significant physiological stress or reductions in body condition.

Amphibian responses to fish vary greatly depending on predator identity and the ecological setting. For example, Kats and Ferrer (2003) [19] reported that predatory fish often trigger strong antipredator responses in amphibians, such as altered behavior or habitat avoidance. Conversely, Hecnar and M'Closkey (1997) [17] found that while predatory fish significantly reduced amphibian species richness and recruitment, non-predatory fish mainly influenced habitat selection rather than directly suppressing amphibian populations through predation. Active predation on amphibian larvae by minnows has not been confirmed, but the ecological overlap between amphibian tadpoles and minnows in shallow littoral zones may lead to incidental interactions, such as nibbling or consumption of smaller or compromised individuals, particularly under resource-limited conditions. Laurila et al. (1997) [22] found that Common frog (*Rana temporaria*) tadpoles exhibited graded antipredator responses based on predator diet, showing significantly stronger avoidance behaviors when exposed to dragonfly larvae that had consumed conspecific tadpoles, suggesting that they can assess and respond to varying levels of predation risk through chemical cues. These patterns suggest that the apparent lack of impact could mask subtle or context-dependent interactions that warrant closer experimental evaluation.

Amphibians have demonstrated the ability to mitigate mild environmental stress through plasticity. Stress-induced physiological adjustments have been observed in multiple species, enabling individuals to maintain normal performance despite environmental challenges. For example, Wilson et al. (2002) [51] reported that African clawed frogs exhibited compensatory trade-offs between speed and endurance in response to different environmental pressures such as habitat desiccation and predation risk. These pressures drove divergent locomotor adaptations, with some individuals prioritizing burst speed for escape responses, while others-maintained endurance to prolonged activity in low-predation or thermally variable environments.

Tadpole survival is influenced by a multitude of ecological and biological factors. For example, Hartel et al. (2007) [16] demonstrated that amphibian species richness was lower in fish-inhabited ponds, suggesting both direct predation effects and behavioural avoidance of fish-occupied habitats. Eaton et al. (2005) [12] further showed that fish presence altered amphibian recruitment dynamics, with indirect effects on population structure.

Conclusions

The results of our study fall in line with previous research showing that amphibians' responses to fish introductions are complex and context-dependent. Although common minnows did not elicit significant carry-over effects on the size, body condition and endurance of *Rana temporaria* metamorphs, their potential to influence early developmental stages—either through indirect mechanisms or opportunistic predation—should not be overlooked. Accordingly, conservation strategies should emphasize the protection of fish-free breeding habitats and consider the ecological implications of introducing or maintaining fish populations in amphibian-rich environments. Notably, empirical evidence demonstrating the rapid recovery of amphibian populations following fish removal (e.g., Bosch et al. 2019[5]) underscores the efficacy of targeted management interventions in preserving biodiversity and ecological integrity, particularly in sensitive habitats such as alpine lakes and ponds.

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