Turkish Journal of Agriculture - Food Science and Technology, 9(sp): 2604-2617, 2021
DOI: https://doi.org/10.24925/turjaf.v9isp.2604-2617.4981

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Effects of Environmentally Relevant Ammonium Nitrate Levels Caused by Agricultural Activities on Four Amphibian Species in The Eastern Black Sea Region[#]

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ARTICLE INFO

[#]*This study was presented as an online presentation at the 2nd International Journal of Agriculture - Food Science and Technology (TURJAF 2021) Gazimağusa/Cyprus*

Research Article

Received : 15/12/2021
Accepted : 31/12/2021

Keywords:
Chronic ammonium
Pelophylax ridibundus
Rana macrocnemis
Pelodytes caucasicus
Bufotes variabilis

ABSTRACT

Within the scope of this research, the chronic effects of ammonium nitrate, which is the most widely used fertilizer worldwide, on the tadpoles of Marsh Frog (*Pelophylax ridibundus*), the Iranian Long-Legged Frog (*Rana macrocnemis*), the Caucasian Parsley Frog (*Pelodytes caucasicus*) and the Variable Green Toad (*Bufotes variabilis*) were examined. To assess the chronic effects of the fertilizer, the tadpoles of all the species were exposed to 0, 5, 10, 15, 20, 25 mg/L concentrations in same conditions. The chronic concentrations were applied from the 25th developmental stage to the 42nd developmental stage. It was defined at the end of the experiments that although there were some variability between different populations of the same species or between different species in the observed effects, chronic levels of ammonium nitrate caused decreased growth rate, prolonging in time to complete metamorphosis, increased abnormalities, and mortality in general. Among the 4 amphibian species, the Variable Green Toad was the most damaged one in terms of growth reduction (on average 31-41 %), abnormality rates (on average 50-75 %), prolonged time to complete metamorphose (14-21 days on average) and mortality rates (%54-100). The most adaptive species and the least damaged one was an Iranian long-legged frog for growth reduction (on average 0-15 %), prolonged time to complete metamorphose (7-9 days on average), and mortality rates (%9-15). All the harmful effects of chronic fertilizer levels caused by agricultural activities in the region had very important for examined species in our researches and it can be said that important environmental and biodiversity problems may occur if certain precautions are not taken regarding the use of the fertilizers and if the attitudes of the farmers on this issue cannot be changed.

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Introduction

Ecosystems are rapidly and globally changing worldwide because of exploitation of the environment by human beings (MEA, 2005; Sage, 2020). It was thought that the exploitation activities will be increase in the future (Pereira et al., 2010). Some results of these activities are the concomitant climate change, emissions of greenhouse effect gases and, biological invasions, modifications of land use inducing habitat fragmentation and loss, and deposition of nitrogen (Sala et al., 2000). Nowadays, the land surface of the Earth (approximately 53%) have been profoundly changed and modified because of increased human population, the profound need for food and agricultural applications (Hooke et al., 2012; Devine and Furlong, 2007). Eventually, the most serious activity of humans is agricultural expansion resulting in "habitat loss" and damaging amphibian populations while considering amphibian population decrease (Gallant et al., 2007; Cordier et al., 2021). Agriculture and agricultural applications are the main reasons for natural ecosystem loss and/or fragmentation (Powell and Lenton, 2013; Hanaˇcek and Rodríguez-Labajos, 2018). Although the agricultural applications can show different characteristics (Waldner et al., 2016), agricultural ecosystems symbolize a main in land use as mentioned above for a source of greenhouse gases, a reservoir of invasive species and a considerable nitrogenous fertilizer input (Miller et al., 2006; Gupta and Khosla, 2012; P´andi et al., 2014; Olivares et al., 2013). Some taxa are more sensitive to agricultural stressors than others (Foden et al., 2008). Among vertebrates, amphibian populations are globally under significant threat (Collins and Crump, 2009), possibly related to the mentioned phenomenon (Sodhi et al., 2008; Whitfield et al. 2017). Besides, the assessment of global amphibian indicated that amphibians was the most threatened organisms among the vertebrate classes with the rate of 41% because of agriculture (IUCN 2021; Harfoot et al., 2021). IUCN (2021) reported that approximately 37 amphibian species have gone globally extinct and many of them were endemic to those particular regions they were found in and 663 species are considered “Critically Endangered”, 1060 are “Endangered”, 721 are “Vulnerable”, and 421 are “Near Threatened” (IUCN 2021). According to the report, approximately the 40% of the 7178 observed species are decreasing in population size showing that more species will become extinct and/or enter higher Red List categories in the future (Beebee and Griffiths 2005, Sodhi et al. 2008). Amphibian populations are frequently endangered by barriers to connectivity and alien competitors, pathogens, predators, and thermal shifts. (Cushman, 2006; Shuman-Goodier et al., 2017; Pounds et al., 2006); Falaschi et al., 2020; Alford et al., 2007). To be susceptible to contamination because of their semi-permeable skins is another disadvantage of amphibians (Egea-Serrano et al., 2012; Slaby et al., 2019). The other major threating factors on amphibian populations are fertilizers and other agrochemicals such as nitrogenous compounds (Krishnamurthy and Smith, 2010; 2011; Trudeau et al., 2020; Van Meter et al., 2019; Marco and Ortiz-Santaliestra, 2009; Baker et al., 2013). If inorganic nitrogen is found at ahigh level in the environment, it may behave as a toxicant and affects the growth and reproduction of mollusks (Alonso and Camargo, 2013; Simplicio et al., 2017), crustaceans (Soucek and Dickinson, 2016), insects (Camargo et al, 2005), amphibians (Marco and Ortiz-Santaliestra, 2009), and fishes (Simplicio et al., 2017; Davidson et al., 2014; McGurk et al., 2006)., crustaceans, insects, amphibians, and fishes. Among them, amphibians are the most threatened class of vertebrates. In other words, the extinction rate of amphibians has exceeded the historic background extinction rate at least by 200 folds in recent decades (Roelants et al. 2007). Nitrogenous compounds damage both the larval and terrestrial stages of amphibian (Marco et al., 2001; Griffis-Kyle, 2007; Daam et al., 2020). However, amphibian larvae cannot escape from the waterbodies they inhabit, and thus they cannot avoid from contamination (Marco et al., 2001). There have been many studies that reported nitrogenous compounds can directly impair tadpole growth and development modify activity levels and lead to decreased survivorship (Karaoglu et al., 2010; Ortiz-Santaliestra et al., 2012; Ilha and Schiesari, 2014; Egea-Serrano et al., 2011; García-Mu˜noz et al., 2011a).

Notable, damaged antipredator attitude is a well-documented harmful secondary effect of nitrogenous compounds on amphibian tadpoles (Ortiz-Santaliestra et al., 2010; Sievers et al., 2018), which could indirectly mediate decreased survivorship (Burgett et al., 2007; Ilha and Schiesari, 2014).

This signifies, some amphibians do thrive in agricultural puddles (Knutson et al., 2004). This is due to tremendous interspecific variation presents among amphibians in sensitivity to nitrogenous compounds (Daam et al., 2020; García-Mu˜noz et al., 2011b; Griffis-Kyle and Ritchie, 2007; Ortiz et al., 2004; Ortiz-Santaliestra et al., 2006;). Furthermore, nitrogenous compounds can be appeared as selective forces capable of developing the improvement of tolerance (Miaud and Meril¨a, 2001). Furthermore, subtraction of groundwater and the foundation of dams, feeding channels and weirs have, in the case of some amphibian species, accidentally supplied breeding habitat where otherwise habitat has been wrecked in agroecosystems and in the lack of less moving predators (i.e., fish) these amphibians insist in the agricultural ecosystems (Knutson et al., 2004; Herzon and Helenius, 2008). Accordingly, geographical divergence in tolerance to nitrogenous compounds has been detected even at the intraspecific level (Shinn et al., 2008), oftentimes as a possible consequence of the differential selective pressures imposed by disparate degrees of exposure (Egea-Serrano et al., 2009; Karaoglu et al., 2010).

Additionally, the effects observed vary according to the nitrogenous compound or the combination of them in question, typically increasing with their concentration (Egea-Serrano et al., 2009, 2011; Egea-Serrano and Tejedo, 2014; Daam et al., 2020). Among these compounds, ammonium may pose a particularly relevant environmental problem in agricultural ecosystems, where it is often particularly common among nitrogenous compounds (Olivares et al., 2013). Ammonium is constantly the dominant nitrogenous compound even in nearby water bodies, following fertilizer leaching (Amano et al., 2016; Liu et al., 2021). Although not without exceptions (Daam et al., 2020), ammonium often exhibits the greatest effects among nitrogenous compounds on traits such as survival, food consumption and growth rate of amphibian larvae (Egea-Serrano et al., 2009; Ilha and Schiesari, 2014; Bellezi et al., 2015; Garriga et al., 2017). Indeed, *Rana sylvatica* tadpoles exposed to nitrate and nitrite do not experience increased mortality (Smith, 2007), whereas those affected by ammonium do (Burgett et al., 2007). Likewise, ammonium has greater effects than nitrite and nitrate on some aspects of the ecology of *Pelophylax perezi* tadpoles, such as heightened activity and food consumption rates, which however results in smaller body size and greater mortality (Egea-Serrano et al., 2009, 2011).

Inorganic nitrogen can have noteworthy physiological effects on amphibians. Nitrate and nitrite have immunosuppressive effects (Camargo and Alonso, 2006), favor the formation of mutagenic and carcinogenic secondary compounds (Nugent et al., 2001), and are speculated to act as endocrine disruptors influencing thyroid function and hormonal balance (Guillette and Edwards, 2005). Nitrite, on top of it, oxidizes hemoglobin into metahemoglobin, a form incapable of transporting oxygen (Huey and Beitinger, 1980), and can affect osmoregulation, neurotransmission, muscle contraction, and heart and mitochondrial function (Camargo and Alonso, 2006). In turn, the ion ammonium is capable of decreasing the internal body concentrations of sodium to fatal levels (Russo, 1985).

As well as direct mortality, these physiological changes have been shown to drive to sublethal effects on individual growth, development, and attitude that could indirectly convert into reduced population vitality (Marco and Ortiz-

Santaliestra, 2009). Slower growth makes tadpoles more sensitive to desiccation and predation because they remain in the aquatic environment for longer periods of time and at susceptible sizes for gape-limited predators (Newman and Dunham, 1994; Wilbur, 1997). But sometimes in some desiccating wetlands, tadpoles respond to pond desiccation via “stress-morphing” and they accelerate the process of their metamorphosis to avoid death (Ruso et al., 2021). However, this acceleration may have negative consequences for immune system function (Gervasi and Foufopoulos, 2008) and mass at metamorphosis of tadpoles (Denver et al. 1998; Gomez-Mestre et al. 2013). Predation risk is also increased because smaller individuals have reduced locomotor capacity (Goater et al., 1993; Beck and Congdon 2000). So, tadpole growth and development are crucial for the individual’s survival and fecundity as an adult (Todd et al. 2011). Slower growth may also reduce future reproductive success because slow growing tadpoles tend to metamorphose at smaller sizes, and smaller metamorphs tend to become smaller adults with lower fecundity and mating success (Smith, 1987; Berven, 1990). In the same way, behavioral fluctuations such as activity rates may be of relevance because activity mediates the tradeoff between foraging and predator avoidance (Werner and Anholt, 1993) and influences the competitive abilities of tadpoles (Semlitsch, 1993; Dayton and Fitzgerald, 2001). Repetitive recruitment failure, either as failure to metamorphose or failure to breed, could result in local population declines or extinctions if conditions are adequately severe (Donald et al. 2011).

Amphibians are unique living beings with extraordinary characteristics that serve people and the environment they live in, although the efficacy are often unknown. As indicator species, the sensitivity of amphibian communities to deteriorating environmental health can be greater than that of the most advanced human-engineered monitoring devices and chemical analyses. (Şişman et al., 2021). Also, they are preferred by scientists as model organism to enlighten the physiology of many different groups of living and human being and they are excellent model organisms for both research and teaching (Wünschmann et al., 2017). Furthermore, by investigating and understanding of their tissue regeneration mechanism, treatments could be developed for humans to heal strokes and spinal cord, eye, brain damage (Brockes and Kumar 2008). Furthermore, amphibians have some skin secretions that act as poison, analgesic, antibiotic agents (Won et al., 2009). Some other secretions inhibit the growth of cancer cells, act as anticancer, antibacterial, antiviral and antifungal agents (Govender et al., 2012). Also, while amphibians are natural predators and controller for invertebrates such as insects and important plant pests such as potato beetles, mole crickets, grasshoppers, and young snails (Attademo et al., 2005-2007; García-Padrón, 2021), they are prey and food for many predator vertebrates in the food chain (Dodd and Barichivich 2007). Thus, they are essential living beings that protect the natural balance of ecosystems. When they disappeared from an ecosystem, they alter many dynamics related to both producers and consumers in the ecosystems they live in (Dodd and Barichivich 2007).

For the current research, we selected 4 amphibian species that are distributed in the Eastern Black Sea Region and stand out with their different characteristics. Chronic effects investigated for the environmentally relevant concentration of ammonium nitrate fertilizer on Marsh Frog (an edible frog that active all-day time), Iranian Long-Legged Frog (a frog that active all day time), Caucasian Parsley Frog (an endemic and nocturnal frog) and Variable Green Toad (a nocturnal toad). Despite the different characteristics, their common point was that, according to the IUCN data, among the different factors threatening each of them, there was the agricultural pollution factor observed at the regional level in some distribution areas.

The Eastern Black Sea Region is the region with the highest rainfall in Turkey and one of the 26 main basins in the country (Eastern Black Sea Basin). Compared to the rich surface water potential supported by small glacial lakes and many small or large rivers, at the underground, groundwater resource potential is weaker due to its extremely sloping topography and geological structure of volcanic rock. There are many aquaculture farms on the rivers within the basin (Öziş et al., 1997; Ak et al., 2008). In addition, in the region, especially around Rize and Trabzon province, tea and hazelnut cultivation is the most intense and agricultural lands are spread over large areas. The farmers in the region, with the logic that the more fertilizers are applied the higher yield will be achieved, are applied approximately 2 or 3 times more fertilizer the values recommended by official agricultural organizations (60-70 kg / ha) and the fertilization applications continuously overlap with precipitation. It is thought that these factors negatively affect the freshwater ecosystems close to this agricultural geography and the main reason triggering nitrogen pollution in the ecosystems originates from existing errors in fertilization applications (Yüksek et al., 2013; Ödün, 2013). According to the results of a study conducted in Fırtına Valley, it was determined that 28-40 % of the applied fertilizers in the region were washed away and leached to water resources, thus caused the pollution of water resources. This confirms the above-mentioned estimates.

In direction with all the reasons above, while current research was being planned, it was aimed to contribute to the protection of biodiversity and amphibians by determining the threshold nitrogen concentrations caused by agricultural activities carried out in the Eastern Black Sea Region which had negative effects on each frog species investigated, and obtaining clues about the future of these species for taking possible required precautions.

Materials and Methods

The Study Sites and Species

Due to the field studies carried out in previous years, the breeding habitats of the selected species were known. According to the results of the water pollution analysis made by DR3900-Hach spectrophotometer, the sampling areas were finalized for obtaining the egg clutches. For all species, two habitats selected with the greatest difference in the nitrogen content (Johansson et al. 2001). Water samples were continued to be collected biweekly throughout the breeding season of the species and analyzed for nitrate, nitrite and ammonium by using the spectrophotometer and the cell kits.

Marsh frog *Pelophylax ridibundus* eggs were collected from the ponds near the Söğütlü River representing the polluted zone and collected from the other ponds near the Değirmendere River representing the natural zone. The Söğütlü River Ponds (41°00'18"N, 39'37'46"E), having maximum depth and maximum diameter respectively of 0,4 m and 2,4 m, is located in an area of intensive agricultural activities. The Değirmendere Ponds (40°59'45"N, 39'46'1 8"E) were a natural small pond with maximum depth and maximum diameter respectively of 0,5 m and 2,7 m. In its surrounding area, agricultural activity is weak. This pond has naturally lower nitrogen content when compared the Söğütlü pond.

Breeding habitats of Iranian Long-legged frog *Rana macrocnemis* located in Akçaabat (Adacık village) and Uzungöl. We chose Uzungöl ponds (40°37'04"N, 40°17'53"E) as the natural habitat. They were some shallow temporary spring ponds located near the helipad and their nitrogen contents were highly low despite the tourism activities surrounding them. Maximum depth of the ponds was 0,26 m and the maximum diameter was 4,1 m. The agricultural h abitat of the Iranian long-legged frog was in Akçaabat (Adacık village 41°00'43"N, 39°33'48" E) river ponds. The maximum depth and diameter of the ponds were respectively 0,7 m and 3,6 m. The ponds were located in some agricultural fields near the Adacık ponds and there were several small ruminant herds surrounded the ponds as possible nitrogen pollution resources. Thus, nitrogen contents of the Adacık ponds were quite high (e.g., 84 mg/L nitrat) compared to all other habitats.

Egg clutches of Caucasian parsley frog *Pelodytes caucasicus* were taken in Hıdırnebi (40°57'27.83''N, 39°26'1.27''E), representing the agricultural habitat, consists of some ponds spread over a wide area, located near a large artificial pond. Some of the ponds that the eggs obtained were temporary, shallow and small ponds formed by rain water on the roadside, but the others were larger ponds with a depth of 1-2 meters and a diameter of 3-5 meters, surrounded by various herbaceous plants. Also, there was an artificial trout aquaculture farm near the study area. As the natural area for the Caucasian parsley frog eggs, we chose Uzungöl habitat (40°36' 44.33'' N, 40°18'42.53''E) located in the backyard of a small motel that isolated from crowded touristic area of Uzungöl. The sampling area were two interconnected ponds located in a large garden covered with grass and some herbaceous plants. While the depth of these ponds was approximately 2 meters, they were about 9 meters in length and 5 meters in diameter. Inside the one of the sampling ponds, there were reeds while inside of the other there were some herbaceous plants. The egg clutches of Caucasian parsley frog in both sampling areas of Hıdırnebi and Uzungöl, were clinging to the plants at the edges of the ponds.

Variable green toad *Bufotes variabilis* egg strings were collected from Artvin (Seyitler Village 41°11'42''N, 41°50'44''E) representing natural habitat and from Akçaabat (Adacık Village 41°01'13''N, 39°34'39''E) representing polluted one. Adacık habitat was the same agricultural area for both Iranian long-legged frog and Variable green toad. However, Variable green toad was generally distributed in the northern parts and shallow ponds of the stream, while Iranian long-legged frog was generally distributed in the more isolated southern parts and deeper ponds of the stream. Artvin (Seyitler village) habitat of the Variable green toad was consist highly shallow temporary ponds with maximum depth and maximum diameter respectively of 0,31 m and 2,9 m in a muddy area. Because agricultural activities were weak around the habitat, Seyitler ponds included highly low nitrogen concentrations. For all selected species, surrounding of the egg sampling ponds covered with some herbaceous plant sometimes including shrubs except for the natural zones of Variable green toad and Iranian long-legged frog. These habitats were located in muddy area as in temporary and highly shallow ponds and surrounding of the ponds not covered with plant. Habitat characteristics are also summarized in Table 1.

Table 1. Some pollution parameter values and some other characteristic of freshwater habitats

	Marsh Frog		Iranian Long-Legged Frog		Caucasian Parsley Frog		Variable Green Frog	
Habitat	Değirmendere	Söğütlü	Uzungöl	Adacık	Uzungöl	Hıdırnebi	Seyitler	Adacık
pH	8.31	8.21	7.67	7.1	7.74	8.36	7.52	6.9
Water Temperature	20-21℃	20-21℃	15-17℃	19-20℃	16-18℃	14-16℃	18-20℃	17-19℃
Altitude	14 m	9 m	1138 m	68 m	1100 m	1600 m	550 m	17 m
Nitrate	0.8	15	1.9	81	1.5	19.9	1.2	84
Nitrite	0.008	0.09	0.027	0.36	0.015	0.16	0.016	0.39
Ammonium	< 2.6	< 2.6	< 2.6	3.0	< 2.6	< 2.6	< 2.6	3.1

Species Chosen for Investigate

Mars Frog **(***Pelophylax ridibundus*): Among the four species, it is the one with the widest distribution in the world and in Turkey. It is also the only species whose population status is ''increasing''. protection status, in terms of the IUCN Red List, it is in the ''Least Concern'' groups. Being an edible frog and being traded for human food, some local populations are endangered. Furthermore, some other local population negatively affects agricultural or industrial pollution (IUCN, 2021).

Iranian Long-Legged Frog (*Rana macrocnemis*) Although its distribution area in the world is not very wide, it has a wide distribution especially in the northern half of Turkey.

Its conservation status in the IUCN list is ''Least Concern''. And its population status is decreasing. Livestock grazing and habitat loss due to agriculture are among its threats for some of their local populations (IUCN, 2021).

Caucasian Parsley Frog (*Pelodytes caucasicus*): For our research, it is important that it is an endemic species to the Caucasus in the world and the Eastern Black Sea region in Turkey. In addition, the fact that it is in the ‘‘Near Threatened’’ species group among the IUCN Red List groups is another reason that requires careful monitoring of this species (IUCN, 2021).

Variable Green Toad (*Bufotes variabilis*): Although its distribution in the world is not very wide, in Turkey it distributes all over the country. Agricultural expansion and pollution are among possible threats in the species while the threatening factors are not known exactly. Its conservation status in the IUCN list is ‘‘Least Concern’’. And its population status is decreasing (IUCN,2021).

Tadpole Raising

From each habitat belonging the four species, 5 to 7 different egg clutches were collected by netting or hand. Then collected eggs transferred to the laboratory. The eggs allowed to hatch in laboratory and the tadpoles were allowed to develop until they reached developmental stage 25 (G25), when external gills completely absorbed (Gosner 1960), in containers filled with reconstituted soft water (RSW) that was made from distilled water and $NaHC0_3$ (48 ma), $CaS0_4.2\ H_20$ (30 mg/L), $MgS0_4.7\ H_20$ (61 mg/L), and KC1 (2 mg). In experiments, a pool of tadpoles from the collected clutches was used.

Chronic Exposure

Within the scope of the current research, it investigated even if there was any interspecific or intraspecific variation in two populations of four species in tolerating the chronic effects of ammonium nitrate fertilizer. Five replicates of six G25 tadpoles from each pond population were exposed to 0, 5, 10, 15, 20, and 25 mg/L NH_4N0_3 until the tadpoles reach to the G42 developmental stage. Experimental concentrations were selected according to the nitrate levels fluctuating in the habitats where the eggs were collected. As recommended in the researches of Johansson et al. (2001) and Hecnar (1995), all selected G25 tadpoles were in similar size and had no abnormalities. This was achieved by weighing each tadpole one by one, taking them into labeled individual containers, and distributing the weighed tadpoles to obtain equal weight averages in all groups with the help of the excel program. So, in the initial weight of the tadpoles did not differ among the replicates or the concentrations (Kruskal Wallis test). The tadpoles were fed daily with boiled lettuce (Hecnar 1995, Marco et al. 1999). The experiment solutions renewed every week and while the solutions were changing, the tadpoles were placed in 1 L jars filled with appropriate concentrations in order to maintain the permanency of test conditions. TRIS-buffer (10 mM) was added to all of the experiment solutions to keep pH an appropriate level at 7,5 (Johansson et al. 2001). While renewing the solutions, all experiment containers were washed with distilled water and rinsed with 70% ethanol to inhibit the growth of nitrifying bacteria and to stabilize nitrates at treatment levels (Hecnar 1995, Johansson et al. 2001).

Each experimental setup had five replicates in 5 L opaque plastic containers and each container had 3 L solution and six tadpoles. Thus, all the concentrations applied totally on 180 tadpoles (6 x 6 x 5) for each pond population. We did not check the nitrogen concentrations of the solutions, but previous studies that were performed similarly demonstrated that ammonium or nitrate concentrations did not differ significantly in one week (Hecnar 1995, Hatch and Blaustein 2000).

In the expeiment, wet weights of the tadpoles were measured at the beginning and at the end of the experiment as mentioned in Johansson et al. (2001). Duration of larval development (from G25 to G42) was recorded at the end of the experiment. Dead animals were recorded and removed daily, and numbers of abnormalities such as swimming slower, reduced feeding, pigmentation loss, delayed responses, paralysis, lordosis, and edema were counted as carried out by Johansson et al. (2001). The same procedure was repeated twice a day for each container (Hecnar 1995; Johanson et al., 2001). The experiments were performed under the same conditions for all the species at 20-21 °C temperature, under natural photoperiod. All experiment solutions were prepared by using RSW explained above.

Statistical Analyses

The nonparametric Kruskal Wallis, Mann-Whitney U, and Chi-Square tests included in the SPSS 22.0 (Statistical Package for Social Science) package program were used to determine whether there was a difference between the growth rates, abnormality rates, mortality rates, and completion time of metamorphosis in the applied experiments. used. Also, Probit analysis (Finney Method [Lognormal Distribution]) included in the StatPlus package program was used to determine the concentration of ammonium nitrate to cause of kill fifty percent of the larval population (LC_{50} 15 days).

Results

At the end of the experiments, the overall effects of chronic ammonium nitrate concentrations were reduced growth, hind limb joint deformities, delay in time to complete metamorphosis, and mortality at a certain rate, although there were some fluctuations in observed deleterious effects among different species studied or between two different populations of the same species. It was determined that these effects occur at higher levels for the clean zone populations than for the polluted one. During the experiments, it was observed that no harmful effects mentioned above did not occur for the control groups tadpoles however, the harmful effects observed in the application groups occurred at significantly higher levels ($P<0.05$) compared to the control group.

Decrease in Growth Rate

It was determined that the growth decreasing rate for Marsh frog (*Pelophylax ridibundus*) tadpoles in the application groups ranged between 16-36% (average 23%) in the polluted area, while it varied between 31-51% (average 42%) in the natural area. The rate of growth reduction was significantly higher in the clean zone compared to the other one ($p=0.003$). For treatment groups belonging to the natural zone, it was approximately 1,7 times higher than the polluted zone (Figure 1-C).

In the application groups of Iranian Long-Legged Frog (*Rana macrocnemis*) tadpoles, there was no decrease in growth for the polluted area population, while the rate of decrease in growth for the clean area population varied between 11-23% (average 15%). So, it can have said that, response of the two population to ammonium nitrate were highly different (Figure 1-B).

It was determined that the rate of growth decrease in Caucasian Parsley Frog (*Pelodytes caucasicus*) tadpoles varied between 0-27% (average 15%) in the polluted area, while it varied between 0-20% (mean 9%) in the clean area, and the difference between the populations was significant (P<0.05). But unexpectedly clean zone population more tolerant in growth to the fertilizer (Figure 1-A).

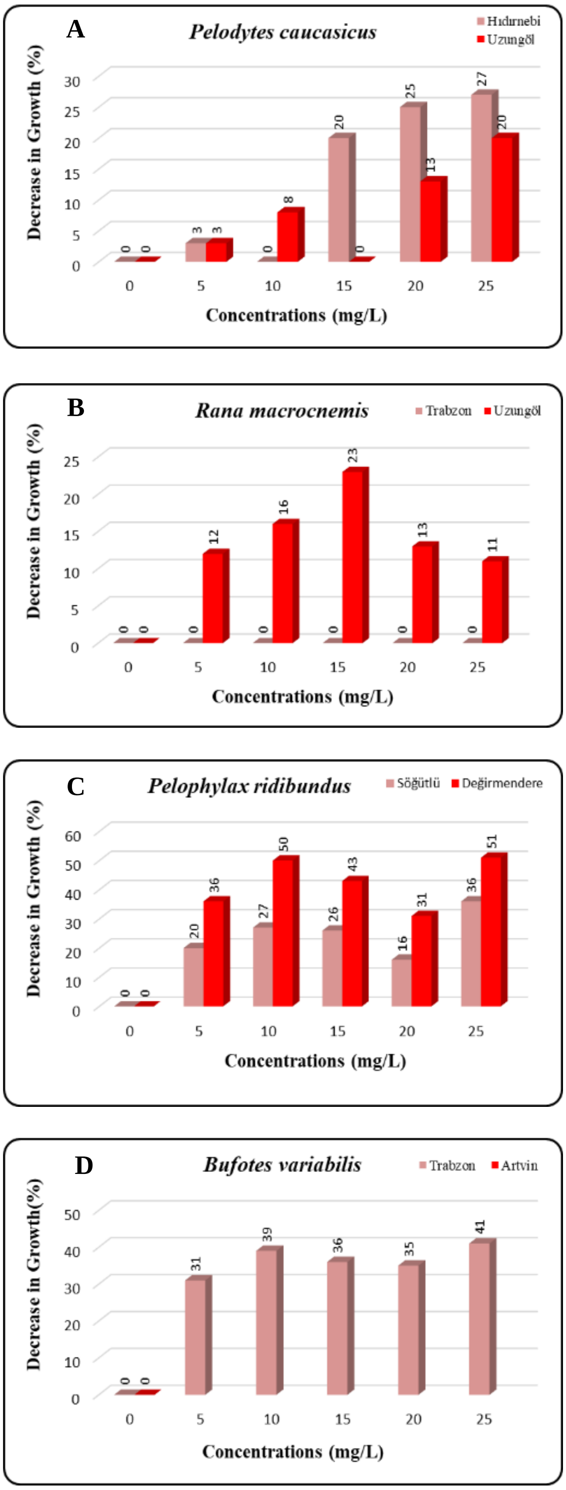

Figure 1. Values of Decrease in Growth.
The red bars represent the values of natural habitat tadpoles and the pink bars represent the values of polluted habitat tadpoles. Each bar represents the avarage values. A) *Pelodytes caucasicus*, B) *Rana macrocnemis*, C) *Pelophylax ridibundus*, D) *Bufotes variabilis*

It was detected that the decrease in growth rate of Variable Green Toad (*Bufotes variabilis*) tadpoles ranged between 31-41% (average 36%) in polluted area. Due to the death of all natural zone tadpoles after the first month of the experiments, difference in the growth of the two populations could not be compared (Figure 1-D).

Among the species examined, the Variable Green Toad was the most damaged species in terms of growth reduction, and the Iranian Long-Legged Frog was the least affected species.

Hind Limb Joints Deformity Rates

The hind limb joints deformity rates of the Marsh frog tadpoles ranged between 19-35% in the polluted area (average 25%), while it ranged between 24-65% in the natural area (average 40%), and the difference for deformity rates in the hind limb joints was found to be significant between the two populations(P<0.05) (Figure 2-C). Some hind limb samples can be seen in Figure 3.

While the hind limb joints deformity rates of the Iranian Long-Legged Frog tadpoles varied between 27-44% in the polluted area (average 38%), the rate of hind limb deformity in the clean area varied between 53-62% (average 57%), and the deformity difference between the two populations was found to be significant (P<0.05) (Figure 2-B).

It was determined that the abnormality rates in Caucasian Parsley Frog tadpoles ranged between 10-57% (average 33%) in the polluted area, while it ranged between 30-59% (average 39%) in the clean area and the difference between the populations was not significant (P>0.05). The abnormalities mentioned here are not hind leg abnormalities but others. Because Caucasian Parsley Frog tadpoles hibernate and do not metamorphose in the year they hatch. Therefore, the ratio of other abnormalities is used to provide comparison data (Figure 2-A).

It was founded that the hind limb joints deformity rates of the Variable Green Toad tadpoles ranged between 50-75% (average 52%) in the polluted area. Since all the tadpoles belong to the clean zone died after the first month of the experiments, two populations could not be compared in terms of this parameter (Figure 2-D).

Among the species we examined, the Variable Green Toad was the most damaged in terms of hind limb joint deformities, and the Mars Frog was the least affected species.

Delay in Time to Complete Metamorphosis

It was observed that the prolongation in the time to complete metamorphosis of Marsh frog tadpoles ranged between 3-10 days (average 7 days) in the polluted area, while it varied between 6-22 days (mean 16 days) in the clean area. It was determined that the prolongation in the time to complete metamorphosis was significantly higher in the natural region compared to the other region (p<0.05) (Figure 4-B).

While the prolongation in the time to complete metamorphosis of Iranian Long-Legged Frog tadpoles varies between 0-13 days (average 6 days) in the polluted region, it varies between 3-13 days (average 10 days) in the clean region, and the prolongation in the completion of metamorphosis was found to be significantly higher in the clean region (Figure 4-A).

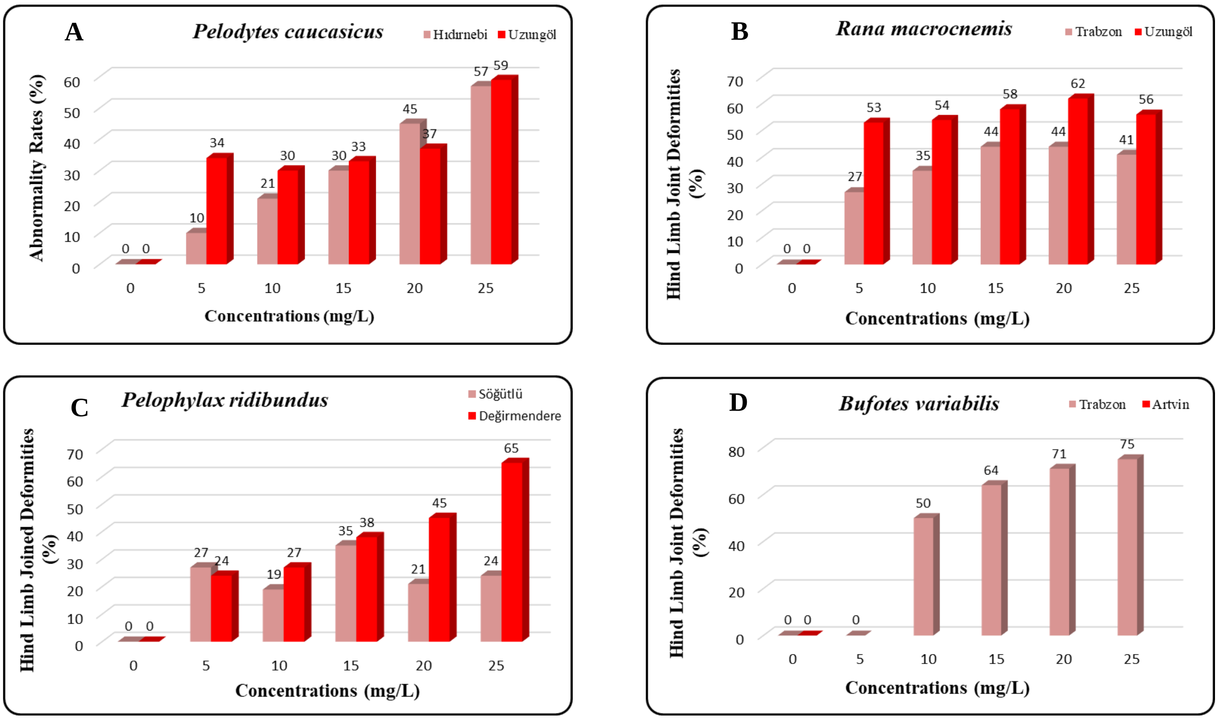

Figure 2. Values of Hind Limb Deformities.
The red bars represent the values of natural habitat tadpoles and the pink bars represent the values of polluted habitat tadpoles. Each bar represents the avarage values. A) Pelodytes caucasicus, B) Rana macrocnemis, C) Pelophylax ridibundus, D) Bufotes variabilis

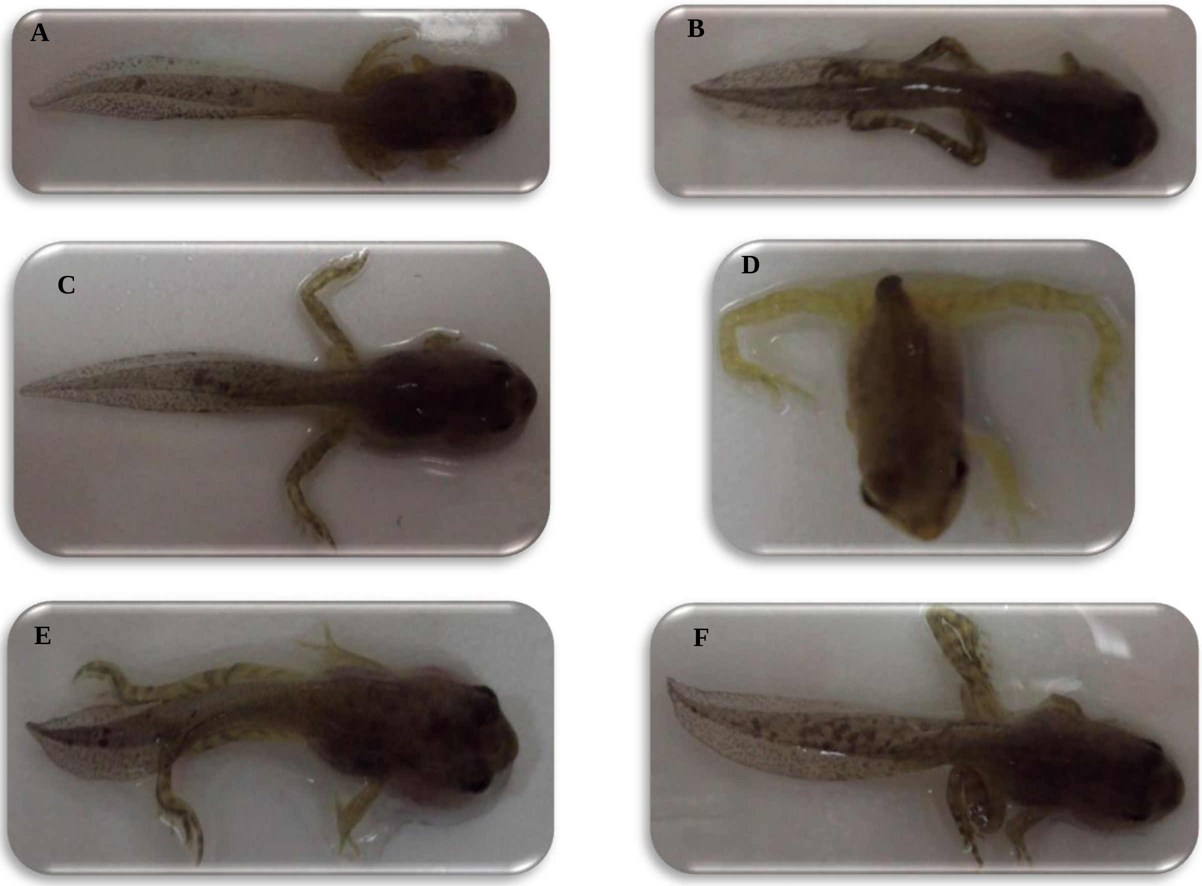

Figure 3. Samples of Different Hind Limb Joint Deformities.
A) Sample of a Healthy Tadpole, B) A Tadpole Sample has Wrist Joint Deformity, C) A Tadpole Sample has Knee Joint Deformity, D) A Metamorph has Knee Joint Deformity, E) A Tadpole Sample has Knee and Hip Joint Deformity, F) A Tadpole Sample has Knee Joint Deformity.

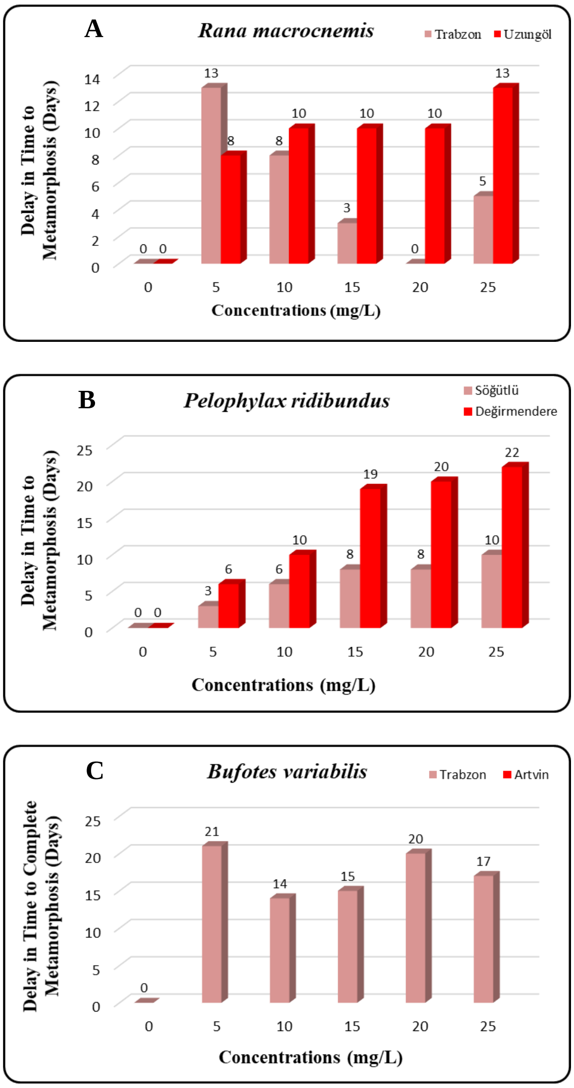

Figure 4. Values of Delay in Time to Metamorphosis.
The red bars represent the values of natural habitat tadpoles and the pink bars represent the values of polluted habitat tadpoles. Each bar represents the average values. A) *Rana macrocnemis*, B) *Pelophylax ridibundus*, C) *Bufotes variabilis*

Because Caucasian Parsley Frog tadpoles hibernate and complete their metamorphosis in the following year summer, these tadpoles could not be evaluated in terms of this parameter.

It was founded that the prolongation in time to complete metamorphosis of the Variable Green Toad tadpoles ranged from 14 to 21 days (mean 18 days) in the polluted area. Since all the tadpoles of the clean zone population died after the first month of the experiments, the two populations could not be compared in terms of this parameter.

Among the species we examined, the Variable Green Toad was the most damaged in terms of prolongation in the metamorphosis period, and the Iranian long-legged frog was determined as the least affected species (Figure 4-C).

Mortality Rates

At the end of the experiment the mortality rates of the Marsh frog tadpoles ranged between 15-45% in the polluted area (average 32%), while it ranged between 45-85% in the clean area (average 66%), and the mortality rates of the clean area were found to be quite significantly high compared to the other area ($P<0.05$) (Figure 5-C).

The mortality rates of Iranian Long-Legged Frog tadpoles at the end of the experiment ranged between 3-27% in the polluted area (8% on average), and it ranged between 10-37% in the clean area (average 15%), and the mortality rates were found to be significantly higher in the clean area population compared to the other population($P<0.05$) (Figure 5-B).

For Caucasian Parsley Frog tadpoles, mortality rates at the end of the experiment ranged between 0-33% in the polluted area (average 21%), while it ranged between 13-47% in the clean area (average 33%), and it was detected that the mortality rates in the clean area population were significantly higher in most groups ($P<0.05$) (Figure 5-A).

It was determined that mortality rates at the end of the experiment for the Variable Green Toad tadpoles varied between 23-77% (average 54) in the polluted area. It was observed that the difference between the two populations was very important in terms of this parameter, since all the clean zone tadpoles died after the first month of the experiments, and the mortality rate in this population was 100% in all treatment groups ($P<0.05$) (Figure 5-D).

Discussion

The findings of a meta-analysis of studies investigating the effects of nitrate on amphibians and fish found that exposure to nitrate reduced survival by 62%, growth 29%, activity 79%, and increased developmental abnormalities by 184%. It also determined that amphibians negatively affect all developmental stages, but show the negative effects most intensely on tadpoles and juveniles. However, adults have reported reduced fertility when exposed to nitrates (Gomez Isaza et al., 2020). The results similar to these findings were confirmed by many studies, including the current study. Therefore, based on all this information, it can be deduced that nitrate compounds are a factor that negatively affects biodiversity, animal health, and the environment in many ways, and it is essential for the world's biodiversity and biotic or abiotic health that they are carefully monitored in nature and their use is strictly regulated.

In the current research, exposure to chronic levels of ammonium nitrate demonstrated significant adverse effects on growth, hind limb health, completing time to metamorphosis for all amphibian species investigated. Furthermore, certain mortality rates were observed in all species studied. If we consider these parameters one by one, we can mention the following results.

The tadpoles of all examined species exhibited a certain reduction in growth. Among the 4 species only Iranian long-legged frogs demonstrate no difference between natural and polluted populations for this parameter. In addition, the growth of the tadpoles belonging to the agricultural habitat did not decrease. But in the case of Marsh frog tadpoles, decreasing growth level reached 51% (average 42%) for population belonging the natural habitat. In a previous study with this species, it was determined that different tolerance levels to ammonium nitrate fertilizer developed in two different populations, and a significant decrease in growth was observed (Karaoglu et al., 2010). But contrary to expectation, nitrate caused increased growth in *Bufo gargarizans* (Wang et al., 2015).

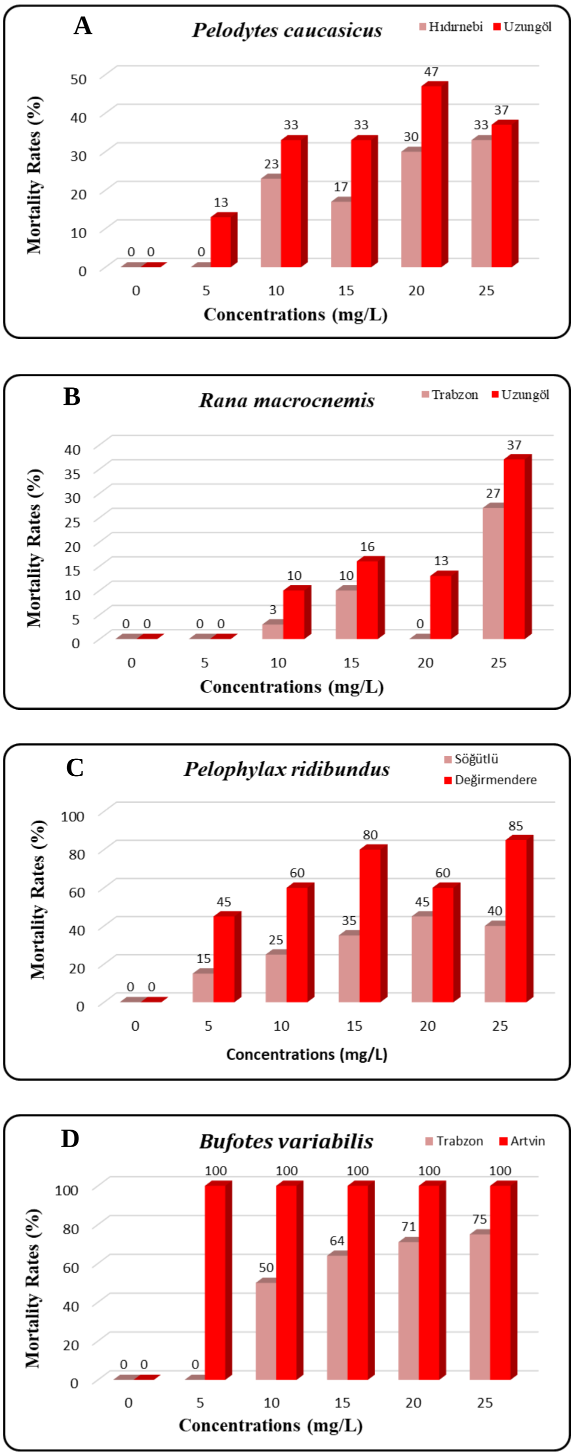

Figure 5. Values of Mortality Rates.
The red bars represent the values of natural habitat tadpoles and the pink bars represent the values of polluted habitat tadpoles. Each bar represents the average values. A) *Pelodytes caucasicus*, B) *Rana macrocnemis*, C) *Pelophylax ridibundus*, D) *Bufotes variabilis*

The trigger for this result is thought to be the prolonged metamorphosis period. Because nitrate prolonged the metamorphosis time of *B. gargarizans.* Unlike current research, Zambrano et al (2022) determined that there was an increase in growth (SVL, tail length, and tail depth) and acceleration in development (further developmental stages) when 10 mg/L ammonium was applied to *P. perezi* tadpoles from natural and agricultural habitats as similar to the study of Smith et al. (2013). In the study of *Lithobates clamitans*, it was seen that ammonium nitrate stimulated growth. In the current study, it was determined that 10mg/L ammonium nitrate reduced growth by an average 20% and delayed the completion of metamorphosis by average 10 days. In the case of *P. perezi*, there was no natural-agricultural population difference in growth reduction and deceleration in development, but in our study, the difference between populations was generally significant. Zambrano et al (2022) determined that only the reduction in swimming speed was significantly greater in the natural zone population than in the agricultural one. This can be considered as evidence that the agricultural region larvae are more resistant, similar to the results of current research. It is thought that the reason why the results of Zambrano et al. (2022) differ from ours may be the thinning in the treatment groups after the deaths in the first week, but the increasing density in the control group may prevent growth. There are different studies showing that density also reduces growth in *Litoria spenceri* and *Litoria aurea* tadpoles (Browne et al., 2003; Gillespie 2002). Also, similar to current research, the growth rate of *Alytes obstetricans* tadpoles decreased at 23 mg/L ammonium over 14 days, but individuals recovered and even increased their growth rate after exposure to the pollutant ended (Garriga et al., 2017) In the current study, 25 mg/L ammonium nitrate caused decreasing growth by mean 27%.

The hazardous changes due to nitrogenous compounds have been shown to lead to sublethal effects on individual growth, development, and behavior that could indirectly translate into reduced population viability (Marco and Ortiz-Santaliestra, 2009). Slower growth makes tadpoles more vulnerable to desiccation and predation because they remain in the aquatic environment for longer periods of time and at vulnerable sizes for gape-limited predators (Newman and Dunham, 1994; Wilbur, 1997). Predation risk is also increased because smaller individuals have reduced locomotor capacity (Goater et al., 1993; Beck and Congdon 2000 Scott and Sloman 2004). So, tadpole growth and development are critical for the individual's survival and fecundity as an adult (Todd et al. 2011). Slower growth may also reduce future reproductive success because slow growing larvae tend to metamorphose at smaller sizes, and smaller metamorphs tend to become smaller adults with lower fecundity and mating success (Smith, 1987; Berven, 1990). Likewise, behavioral changes such as activity rates may be of relevance because activity mediates the tradeoff between foraging and predator avoidance (Werner and Anholt, 1993) and influences the competitive abilities of tadpoles (Semlitsch, 1993; Dayton and Fitzgerald, 2001). Repetitive recruitment failure, either as failure to metamorphose or failure to breed, could result in local population declines or extinctions if conditions are sufficiently severe (Donald et al. 2011).

Chronic levels of ammonium nitrate also caused hind limb joint deformities of all the species examined. Only in the Caucasian parsley frog this parameter could not be evaluated because the tadpoles of this species hibernate and undergo metamorphose the following year. For this reason, the rates of observed other abnormalities such as slowdown and disequilibrium in swimming and decrease in feeding were used to give an idea, albeit partially, in this species. For the other 3 species, hind limb deformities were observed at a significantly higher rate in natural habitats than in agricultural habitats, reaching level of 75% in Variable Green Toad tadpoles. Although not associated with agricultural pollution, Zambrano et al (2020) found hind limb joint deformities in some tadpoles of *P. perezi*. Ammonium did not increase the hind limb abnormality compared to the clean zone. But *P. perezi* is a common sight in agrosystems (Clavero et al., 2015; Carpio et al., 2016), therefore, it may have developed an adaptation to agricultural pollution. This is probably why the proportion of limb abnormalities was the same in the two populations of *P. perezi*. It was determined that hind limb abnormalities did not increase in *Rana temporaria* when compared to the reference regions in agricultural areas (Piha et al., 2006). However, there is evidence that being close to agricultural areas increases the prevalence of limb abnormalities in some anurans (Burghelea et al., 2013; Guerra and Aráoz, 2016; Hedge et al., 2019).

Since functional limbs are very important for vital activities such as movement, hunting and escaping from predators, and individuals with poor mobility skills are rapidly eliminated due to hunting or hunger due to not being able to hunt the malformed limb frequency is kept low (%5) by natural selection (Mester et al., 2015). Supporting this information, it has been proven that the chances of surviving predation attacks by fish (Teplitsky et al., 2005), turtles (Feder, 1983), dragonflies (Chovanec, 1992), snakes (Watkins, 1996) and other tadpoles (Arendt, 2009) are higher for the tadpoles which are faster swimmers than others.

In the current study, hind limb deformities varied between 25% (highly different from the natural prevalence 5%) even at 5 mg/L ammonium nitrate concentration. Therefore, it can be easily said that the cause of joint injuries at the end of the study is ammonium nitrate.

Another adverse effect of chronic concentrations of ammonium nitrate for the studied species was the prolongation of the metamorphosis time. While it could not be evaluated for the above-mentioned reason in the Caucasian parsley frog, a certain prolongation in metamorphosis times was observed in the tadpoles of all other species, and it was observed that it was significantly longer in clean habitat populations than in polluted ones. Similar to the results of the current research, 10, 50 and 100 mg/L nitrate caused elongation to complete metamorphosis of *Bufo gargarizans* tadpoles. At the end of the experiments (78th day), the percentage of metamorphosis of the tadpoles belonging 0, 10, 50 ,100 mg/L groups were 50%, 45%, 24%, 37% respectively and in the 50 and 100 mg/L groups the elongation were significantly higher than the group of control similar to the current study (Wang et al., 2015). In the current study, the duration of metamorphosis was prolonged by an average of 10 days at 10 mg/L and by an average of 14 days at 25 mg/L. This elongations were significantly higher than that of control group.

Elongation in the time to complete metamorphosis makes tadpoles more vulnerable to desiccation and predation because they remain in the aquatic environment for longer periods of time (Newman and Dunham, 1994; Wilbur, 1997). But occasionally tadpoles respond to adverse conditions such as pond desiccation or infections via “stress-morphing” and they accelerate the process of their metamorphosis to avoid death risk (Lent and Babbitt 2020; Ruso et al., 2021; Warne et al., 2011). However, in the case of exposure to ammonium nitrate, this system does not work because ammonium nitrate is a thyroid endocrine suppressor, so metamorphosis cannot be accelerated, but rather lengthens in the process (Wang et al., 2015).

The tadpoles belonging to 100 mg/L nitrate exhibited significant reduction in thyroid hormone levels (T3 and T4). Moreover, treatment with nitrate altered the regulation of mRNA levels of thyroid hormons demontsrating the disruption of thyroid endocrine. Furthermore, 100 mg/L nitrate caused in the thyroid gland follicles local colloid depletion impairing TH synthesis exhibited significant differentiation from control groups. Consequently, ıt can be said that nitrate can act as a chemical stressor triggering prolongation in development and metamorphosis (Wang et al., 2015).

Exposure to chronic concentrations of ammonium nitrate caused certain levels of mortality in the larvae of all species studied. In particular, the native population of the *Bufotes variabilis* species showed 100% mortality in all treatment groups, including concentrations of 5 mg/L. It was thought that the mortalities could be caused by an error made in the experiment process without realizing it, although the same result was obtained 2 times when the experiments repeated second times, confirming that the situation was due to the sensitivity of the species. It was observed that the mortality cases occurred in clean habitats of all species were approximately 2 times higher than the agricultural habitats, and there was a significant difference between two habitat populations. Similar to current study findings, ammonium concentrations above 34 mg/L caused mortalities in Alytes obstetricans larvae in 14 days (Garriga et al., 2017) and *Bufo gargarizans* tadpoles at 10-50-100 mg/L nitrat in 78 days. Mortality rates of control (0), 10, 50, and 100 mg/L nitrate were 4%, 5%, 13% and 25%, respectively. And mortalities of groups exposed to 50 and 100 mg/L nitrate were significantly higher than that of control groups. In the current study mean mortality rates at 10 and 25 mg/L nitrate were 26% and 42% respectively differently from control groups.

In addition, in *P. perezi* tadpoles Zambrano et al (2022) determined that, similar to the results observed by Griffis-Kyle and Ritchie (2007) in *Rana sylvatica* tadpoles, ammonium increased larval mortality only in the first week (only in the early period) after hatching and remained constant in the following weeks. In our study, it was observed that the mortalities increased in parallel with the increase in time. It is thought that this difference may be due to the difference in tolerance between species. It is known that different species have different tolerance levels to ammonium nitrate (Egea-Serrano et al., 2012; Karaoglu. 2011; Karaoglu et al., 2010; Mann et al., 2009). According to Egea Serrano et al. (2009), it determined that 14 mg/L ammonium caused approximately 70% mortality in *Pelophylax perezi* tadpoles at the end of the experiment similar to our findings. In our study, when all populations were evaluated, it was observed that an average of 45% mortality occurred at 15 mg/L.

Nitrogen pollution is recognized as a strong pressure factor on amphibians, and exposure to different nitrogen pollution conditions in different geography can lead to the development of intraspecific adaptation, as evidenced by fluctuations in observed damages among the species studied (Shinn et al., 2008; Karaoglu, 2011; Karaoglu et al., 2010).

Pristine amphibian habitats such as forests were transformed to agricultural areas and concentrated production with intensive application of fertilizers and pesticides in these areas threatenes amphibians obligatory inhabiting there. It documented in the coffee plantations that, 15 amphibian species inhabit in NPK applied field, 19 amphibian species inhabit in organic fields whit no artificial chemical applied and 13 amphibian species inhabit in fields pesticide applied fields. Consequently, agrochemicals and agricultural activities affect negatively amphibian popuations. (Rathod and Rathod 2013). Hegde et al (2019) and Zvelev et al (2014; 2015) reported that such polluted plantations with agrochemicals increased hepatosomatic index (HSI), gonadosomatic index (GI) and morphological abnormalities, decreased concentrations of acetylcholinesterase levels. Adversely affected gonads, brains, livers and acetylcholinesterase associated with increased morphological abnormalities and altered biological functions. It caused to hinder normal growth and healthy biological functions. Therefore, these harmful effects have potential to decrease richness and abundance the amphibians.

In the light of the current research results, it was defined that although there was some variability between two different populations of same species or among four different species in the observed harmful effects, chronic levels of ammonium nitrate caused decreased growth rate, prolonging in time to complete metamorphosis, increased limb abnormalities and mortalities in general. Agricultural activities raised nitrate concentrations in polluted habitats approximately 20 and 80 mg/L. The mean detrimental effects of 20 mg/L ammonium nitrate across all populations were 19% reduction in growth, 46% hind limb abnormality, 12 days delay in metamorphosis, and 46% mortality. These are highly striking damages for the future population status and richness the amphibians studied because of the high mortality risk due to reduction growth and malformed hind limbs. Consequently, it can be said that important biodiversity loss may occur if certain precautions are not taken regarding the use of the fertilizers and if the attitudes of the farmers about false using fertilizers cannot be changed.

References

Ak O, Çakmak E, Aksungur M, Çavdar Y, Zengin B. 2008. Akarsu Üzerindeki Doğal ve İnsan Kaynaklı Faaliyetlerin Sucul Ekosisteme Etkisine Bir Örnek: Yanbolu Deresi (Arsin, Trabzon). Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 24**:** 1-2, 389-400.

Alford RA, Bradfield KS, Richards SJ. 2007. Global warming and amphibian losses. Nature, 447, E3-E4.

Amano H, Nakagawa K, Berndtsson R. 2016. Groundwater geochemistry of a nitrate-contaminated agricultural site. Environ. Earth Sci., 75: 1145.

Arendt J. 2009. Influence of sprint speed and body size on predator avoidance in New Mexican spadefoot toads (Spea multiplicata). Behav. Ecol., 159: 455-461.

Attademo A, Peltzer PM, Lajmanovich RC. 2007. Feeding habits of Physalaemus biligonigerus (*Anura*, *Leptodactylidae*) from soybean field of Córdoba province, Argentina. Russian Journal of Herpetology, 14(1): 1-6.

Attademo AM, Peltzer PM, Lajamanovich RC. 2005 Amphibians occurring in soybean and implications for biological control in Argentina. Agriculture, Ecosystems and Environment, 106: 389–394.

Baker NJ, Bancroft BA, Garcia TS. 2013. A meta-analysis of the effects of pesticides and fertilizers on survival and growth of amphibians. Sci. Total Environ., 449: 150-156.

Beck CW, Congdon JD. 2000. Effects of age and size at metamorphosis on performance and metabolic rates ofSouthern Toad, Bufo terrestris, metamorphs. Funct Ecol., 14:32-8

Beebee TJC, Griffiths RA. 2005. The amphibian decline crisis: a watershed for conservation biology? Biol. Conserv, 125: 271-285.

Bellezi L, Ilha P, Schiesari L. 2015. Ontogenetic variation in the sensitivity of the gladiator frog, Hypsiboas faber, to inorganic nitrogen. Copei, 103: 14–21.

Berven, K. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (Rana sylvatica). Ecology, 71:1599-1608.

Brockes JP, Kumar A. 2008.Comparative aspects of animal regeneration. Annu Rev Cell Dev Biol.,24:525-49.

Browne RK, Pomering M, Hamer AJ. 2003. High density effects on the growth, development and survival of Litoria aurea tadpoles. Aquaculture. 215: 109-121.

Burgett AA, Wright CD, Smith GR, Fortune DT, Johnson SL. 2007. Impact of ammonium nitrate on wood frog (*Rana sylvatica*) tadpoles: effects on survivorship and behavior. Herpetol. Conserv. Biol., 2: 29–34.

Burghelea C, Zaharescum D, Palanca A. 2013. Phenotypic indicators of developmental instability in an endemic amphibian from an altered landscape (Monegros, NE Spain). Amphib-Reptil., 34: 505-516.

Camargo JA, Alonso A, Salamanca A. 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere, 58: 1255-1267.

Camargo JA, Alonso A. 2006. Ecological andtoxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environment International, 32: 831-849.

Carpio AJ, Oteros J, Tortosa FS, Guerrero-Casado J. 2016. Land use and biodiversity patterns of the herpetofauna: the role of olive groves. Acta Oecol., 70: 103-111.

Chovanec A. 1992. The influence of tadpole swimming behaviour on predation by dragonfly nymphs. Amphibia-Reptilia, 13: 341-349.

Clavero M, L´opez V, Franch N, Pou-Rovira Q, Queral JM. 2015. Use of seasonally flooded rice fields by fish and crayfish in a Mediterranean wetland. Agric. Ecosyst. Environ., 213: 39-46.

Collins JP, Crump ML. 2009. Extinction in Our Times: Global Amphibian Decline. Oxford University Press, New York, New York, USA.

Cordier JM, Aguilar R, Lescano JN, Leynaud GC, Bonino A, Miloch D, Loyola R, Nori J. 2021. A global assessment of amphibian and reptile responses to land-use changes, Biological Conservation, 253: 1-10

Cushman SA. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biol. Conserv., 128: 231–240.

Daam MA, Ilha P, Schiesari L. 2020. Acute toxicity of inorganic nitrogen (ammonium, nitrate and nitrite) to tadpoles of five tropical amphibian species. Ecotoxicology. 29: 1516-1521.

Davidson J, Good C, Welsh C, Summerfelt ST. 2014. Comparing the effects of high vs. low nitrate on the health, performance, and welfare of juvenile rainbow trout Oncorhynchus mykiss within water recirculating aquaculture systems. Aquacult. Eng., 59: 30-40.

Dayton GH, Fitzgerald LA. 2001. Competition, predation, and the distributions of four desert anurans. Oecologia, 129: 430-435.

Denver RJ. 1998. Hormonal Correlates of Environmentally Induced Metamorphosis in the Western Spadefoot Toad,Scaphiopus hammondii.General and Comparative Endocrinology, 110(3): 326-336.

Devine GJ, Furlong, MJ. 2007. Insecticide use: contextsand ecological consequences. Agriculture and HumanValues, v.24, p.281-306, 2007.

Dodd CK, Barichivich WJ. 2007. Establishing a Baseline and Faunal History in Amphibian Monitoring Programs: The Amphibians of Harris Neck, GA. Southeastern Naturalist, 6:125.

Donald DB, Aitken WT, Paquette C, Wulff SS. 2011. Winter snowfall determines the occupancy of northern prairie wetlands by tadpoles of the wood frog (Lithobates sylvaticus). Can. J. Zool., 89: 1063-1073.

Egea-Serrano A, Tejedo M, Torralva M. 2009. Populational divergence in the impact of three nitrogenous compounds and their combination on larvae of the frog Pelophylax perezi (Seoane, 1885). Chemosphere, 76: 869-877.

Egea-Serrano A, Tejedo M, Torralva M. 2011. Behavioral responses of the Iberian waterfrog, Pelophylax perezi (Seoane, 1885), to three nitrogenous compounds in laboratory conditions. Ecotoxicology, 20: 1246-1257.

Egea-Serrano A, Tejedo M. 2014. Contrasting effects of nitrogenous pollution on fitness and swimming performance of Iberian waterfrog, Pelophylax perezi (Seoane, 1885), larvae in mesocosms and field enclosures. Aquat. Toxicol., 146: 144-153.

Falaschi M, Melotto A, Manenti R, Ficetola GF. 2020. Invasive species and amphibian conservation. Herpetologica, 76: 216-227.

Feder ME. 1983. The relation of air breathing and locomotion to predation on tadpoles, Rana berlandieri, by turtles. Physiol. Zool., 56: 522-531.

Foden, W, Mace G, Vi´e, J.C, Angulo A, Butchart S, DeVantier L, Dublin H, Gutsche A, Stuart S, Turak E. 2008. Species susceptibility to climate change impacts. In: Vi´e, J.C., Hilton-Taylor, C., Stuart, S.N. (Eds.), The 2008 Review of the IUCN Red List of Threatened Species. IUCN, Gland, Switzerland, pp., 77–88.

Gallant AL, Klaver RW, Casper GS, Lannoo MJ. 2007. Global rates of habitat loss and implications for amphibian conservation. Copeia, 2007:967-979.

García-Mu˜noz E, Guerrero F, Bicho RC, Parra G. 2011b. Effects of ammonium nitrate on larval survival and growth of four Iberian amphibians. Bull. Environ. Contam. Toxicol., 87: 16-20.

García-Mu˜noz E, Guerrero F, Parra G. 2011a. Effects of previous sublethal pulse to ammonium nitrate on mortality and total length on Epidalea calamita larvae. Chemosphere, 84: 671-675.

García-Padrón LY. 2021. Diet of a community of frogs in an agroecosystem in western Cuba Caribbean Herpetology, 76: 1–8.

Garriga N, Montori A, Llorente G.A. 2017. Impact of ammonium nitrate and sodium nitrate on tadpoles of Alytes obstetricans. Ecotoxicology, 26: 667-674.

Gervasi SS, Foufopoulos J. 2008. Costs of plasticity: Responses to desiccation decrease post-metamorphic immune function in a pond-breeding amphibian. Functional Ecology, 22(1):100 - 108

Gillespie GR. 2002. Impacts of sediment loads, tadpole density, and food type on the growth and development of tadpoles of the spotted tree frog Litoria spenceri: an in-stream experiment. Biol. Conserv., 106: 414-150.

Goater CP, Semlitsch RD, Bernasconi M. 1993. Effects of body size and parasite infection on the locomotory performance of juvenile toads Bufo bufo. Oikos, 66: 129-136.

Gomez Isaza DF, Cramp RL, Franklin CE. 2018. Negative impacts of elevated nitrate on physiological performance are not exacerbated by low pH. Aquat. Toxicol., 200: 217-225.

Gomez Isaza, DF., Cramp, RL, Franklin, CE, 2020., Living in polluted waters: A meta-analysis of the effects of nitrate and interactions with other environmental stressors on freshwater taxa, Environmental Pollution, 261: 114091-114502.

Gomez-Mestre I, Kulkarni S, Buchholz DR. 2013. Mechanisms and Consequences of Developmental Acceleration in Tadpoles Responding to Pond Drying. PLoS ONE, 8(12): e84266.

Gosner K. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica, 16: 183-190.

Govender T, Dawood A, Esterhuyse AJ, Katerere DR. 2012. Antimicrobial properties of the skin secretions of frogs. South African Journal of Science, 108(5-6):25-30

Griffis-Kyle KL, Ritchie, ME. 2007. Amphibian survival, growth and development in response to mineral nitrogen exposure and predator cues in the field: an experimental approach. Oecologia, 152: 633-642.

Griffis-Kyle KL. 2007. Sublethal effects of nitrite on eastern tiger salamander (Ambystoma tigrinum tigrinum) and wood frog (Rana sylvatica) embryos and larvae: implications for field populations. Aquat. Toxicol., 41: 119-127.

Guerra C, Aráoz E. 2016. Amphibian malformations and body condition across an agricultural landscape of northwest Argentina. Dis. Aquat. Organ., 121: 105-116.

Guillette LJ, Edwards TM. 2005. Is nitrate an ecologically relevant endocrine disruptor in vertebrates? Integr. Comp. Biol.,45(1):19-27.

Gupta ML, Khosla R, Precision Nitrogen Management and Global Nitrogen Use Eficiency, 11th International Conference on Precision Agriculture, Indianapolis, Indiana, 2012.

Hanaˇcek, K, Rodríguez-Labajos B. 2018. Impacts of land-use and management changes on cultural agroecosystem services and environmental conflicts – a global review. Global Environ. Change 50: 41-59.

Harfoot MBJ, Johnston A, Balmford A, Burgess ND, Butchart SHM, Dias MP, Hazin C, Hilton-Taylor C, et al. 2021. Using the IUCN Red List to map threats to terrestrial vertebrates at global scale. Nature Ecology & Evolution.

Hatch AC, Blaustein AR. 2000. Combined effects of UV-B, nitrate and low pH reduce the survival and activity level of larval Cascades frogs (Rana cascadae). Archives of Environmental Contamination and Toxicology, 39:494-499.

Hecnar S., 1995. Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from southern Ontario. Environ. Toxicol. Chem., 14: 2131-2137.

Hegde G, Krishnamurthy SV, Berger G. 2019. Common frogsresponse to agrochemicals contamination in coffee plantations, WesternGhats, India. Chemistry and Ecology,35(5): 397–407

Herzon I, Helenius J. 2008. Agricultural drainage ditches, their biological importance and functioning. Biological Conservation, 141(5):1171-1183

Hooke RLB, Martín-Duque JF, Pedraza J. 2012. Land transformation by humans: A review. GSA Today, 22: 4–10.

Huey DW, Beitinger TL. 1980. Hematological responses of larval Rana catesbeiana to sublethal nitrite exposures. Bulletin of Environmental Contamination and Toxicology, 25: 574-577.

Ilha P, Schiesari L, 2014. Lethal and sublethal effects of inorganic nitrogen on gladiator frog tadpoles (Hypsiboas faber, Hylidae). Copeia, 221–230.

IUCN 2021. IUCN (International Union for Conservation of Nature and Natural Resources). https://www.iucnredlist.org/search?query=Amphibians&searchType=species

IUCN, 2020. The IUCN Red List of Threatened Species. International Union for Conservation of Nature and Natural Resources. https://www. iucnredlist.org.

Johansson M, Ra¨sa¨nen K, Merila¨ J. 2001. Comparisonof nitrate tolerance between different populations of the common frog, Rana temporaria. Aquatic Toxicology, 54: 1-14.

Karaoglu H, Kutrup B, Ogut H. 2010. Intraspecific differences in responses of marsh frog (Pelophylax ridibundus) tadpoles exposed to environmentally relevant and acute levels of ammonium nitrate fertilizers. J. Freshw. Ecol., 25: 353-366.

Karaoglu H. 2011.Amonyum nitrat suni gübresinin Kafkas Kurbağası (Pelodytes caucasicus) larvaları üzerindeki akut ve kronik etkileri, Doktora tezi, Karadeniz Teknik Üniversitesi, Fen Bilimleri Enstitüsü, Trabzon, Türkiye.

Knutson MG, Richardson WB, Reineke DM, Gray BR, Parmelee JR, Weick SE. 2004. Agricultural ponds support amphibian populations. Ecol. Appl., 14: 669-684.

Krishnamurthy SV, Smith GR. 2010.Growth, abnormalities, and mortality of tadpoles of American toad exposed to combinations of malathion and nitrate. Environ Toxicol Chem., 29: 2777-2782.

Lent EM, Babbitt KJ. 2020. The effects of hydroperiod and predator density on growth, development, and morphology of wood frogs (Rana sylvatica). Aquat. Ecol., 54; 369-386.

Liu XL, Han G, Zeng J, Liu M, Li XQ, Boeckx P. 2021. Identifying the sources of nitrate contamination using a combined dual isotope, chemical and Bayesian model approach in a tropical agricultural river: case study in the Mun River, Thailand. Sci. Total Environ., 760, 143938.

loss and implications for amphibian conservation. Copeia 2007:967–979

Marco A, Cash D, Belden LK, Blaustein AR. 2001. Sensitivity to urea fertilization in three amphibian species. Arch. Environ. Contam. Toxicol., 40: 406-409.

Marco A, Cash D, Belden LK., Blaustein AR. 2001. Sensitivity to urea fertilization in three amphibian species. Arch. Environ. Contam. Toxicol., 40: 406-409.

Marco A, Ortiz-Santaliestra M.E. 2009. Impact of reactive nitrogen on amphibians. In: Heatwole, H., Wilkinson, J.W. (Eds.), Amphibian Decline: Diseases, Parasites, Maladies and Pollution, vol. 8. Surrey Beatty & Sons, Baulkham Hills, New South Wales, Australia. Amphibian Biology.

Marco A, Quilchano C, Blaustein AR. 1999. Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest. Environmental Toxicology and Chemistry, 18: 2836-2839.

McGurk MD, Landry F, Tang A, Hanks, CC. 2006. Acute and chronic toxicity of nitrate to early life stages of lake trout (Salvelinus namaycush) and lake whitefish (Coregonus clupeaformis). Environ. Toxicol. Chem., 25: 2187-2196.

McKenzie DJ, Shingles A, Claireaux G, Domenici P. 2009. Sublethal concentrations of ammonia impair performance of the Teleost fast-start escape response. Physiol Biochem Zool, 82: 353-362.

MEA (Millennium Ecosystem Assessment). 2005. Ecosystems and Human Well-Being: Scenarios. Island Press, Washington DC.

Mester B, Lengyel S, Puky M. 2015. Low frequency of amphibian morphological anomalies in a large protected wetland and grassland complex in Hungary. Herpetol Conserv Biol.,10: 679–687.

Miaud C, Meril¨a J. 2001. Local adaptation or environmental induction? Causes of population differentiation in alpine amphibians. Biota, 2:31–50.

Miller SA, Landis AE, Theis TL. 2006. Use of Monte Carlo analysis to characterize nitrogen fluxes in agroecosystems. Environ. Sci. Technol, 40: 2324-2332.

Monsees, H, Klatt L, Kloas W, Wuertz S. 2017. Chronic exposure to nitrate significantly reduces growth and affects the health status of juvenile Nile tilapia (*Oreochromis niloticus L.*) in recirculating aquaculture systems. Aquacult. Res., 48: 3482-3492.

Newman RA, Dunham AE. 1994. Size at metamorphosis and water loss in a desert anuran (Scaphiopus couchii). Copeia, 1994: 372-381.

Nugent, M, Kamrin MA, Wolfson L., D'Itri FM. 2001. Nitrate: a drinking water concern. Michigan State University Extension Service, Extension bulletin WQ-19. http://www.msue.msu.edu/objects/content_revision/download.cfm/revision_id.499674/workspace_id.-4/01500617.html. Accessed 18 September 2006.

Ödün NA. 2013. Fırtına Vadisi'nde (Çamlıhemşin-Rize) Çay Tarımında Kullanılan Suni ve Doğal Gübrelerin Oluşturduğu Su Kirliliğinin Akuatik Ekosisteme Etkisi. Recep Tayyip Erdoğan Üniversitesi, Fen Bilimleri Enstitüsü, Rize,Türkiye.

Olivares J, Bedmar, EJ, Sanju´an J. 2013. Biological nitrogen fixation in the context of global change. Mol. Plant Microbe Interact, 26: 486-494.

Ortiz ME, Marco A, Saiz N, Lizana M. 2004. Impact of ammonium nitrate on growth and survival of six European amphibians. Arch. Environ. Contam. Toxicol., 47: 234-239.

Ortiz-Santaliestra ME, Fern´andez-Ben´eitez MJ, Marco A, Lizana M. 2010. Influence of ammonium nitrate on larval anti-predatory responses of two amphibian species. Aquat. Toxicol., 99: 198-204.

Ortiz-Santaliestra ME, Fern´andez-Ben´eitez MJ, Marco A. 2012. Density effects on ammonium nitrate toxicity on larval amphibians. Survival, growth and cannibalism. Aquat. Toxicol., 110–111, 170–176.

Ortiz-Santaliestra ME, Marco A, Fern´andez MJ, Lizana M. 2006. Influence of developmental stage on sensitivity to ammonium nitrate of aquatic stages of amphibians. Environ. Toxicol. Chem., 25: 105-111.

Öziş Ü, Baran T, Durnabaşı İ, Özdemir Y. 1997. Türkiye'nin Su Kaynakları Potansiyeli. Meteoroloji Mühendisliği, TMMOB Meteoroloji Mühendisleri Odası Yayın Organı, 2; 40-45.

P´andi I, Penksza K, Botta-Duk´at Z, Kr¨oel-Dulay G. 2014. People move but cultivated plants stay: abandoned farmsteads support the persistence and spread of alien plants. Biodivers. Conserv, 23: 1289-1302.

Pereira HM, Leadley PW, Proença V, Alkemade R, Scharlemann JPW, Fern´andez-Manjarr´es JF, Araújo MB, Balvanera P, Biggs R, Cheung WWL, Chini L, Cooper HD, Gilman EL, Gu´enette S, Hurtt GC, Huntington HP, Mace GM, Oberdorff T, Revenga C, Rodrigues P, Scholes RJ, Sumaila US, Walpole M. 2010. Scenarios for global biodiversity in the 21st century. Science, 330: 1496-1501.

Piha H, Pekkonen M, Merilä, J. 2006. Morphological abnormalities in amphibians in agricultural habitats: a case study of the common frog Rana temporaria. Copeia, 2006: 810-817.

Pounds JA, Bustamante MR, Coloma LA, Consuegra JA, Fogden MPL, Foster PN, La Marca E, Masters KL, Merino-Viteri A, Puschendorf R, Ron SR, S´anchez-Azofeifa GA, Still CJ, Young BE. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature, 439: 161-167.

Powell TWR, Lenton TM. 2013. Scenarios for future biodiversity loss due to multiple drivers reveal conflict between mitigating climate change and preserving biodiversity. Environ Res Lett., 8: 025024.

Rathod S, Rathod P. 2013.Amphibian communities in three different coffee plantation regimes in the Western Ghats. India. J Threat Taxa., 5: 4404-4413.

Roelants K, Gower DJ, Wilkinson M, Loader PS, Biju SD, Guillaume K, Moriau L, Bossuyt F. 2007. Global patterns of diversification in the history of modern amphibians. Pnas, 104,3: 887-892.

Ruso GE, Hogan NS, Sheedy C, Gallant MJ, Jardine TD. 2021. Effects of Agricultural Stressors on Growth and an Immune Status Indicator in Wood Frog (Lithobates sylvaticus) Tadpoles and Metamorphs. Environmental Toxicology and Chemistry, 40(8): 2269-2281.

Russo RC. 1985. Ammonia, nitrite and nitrate, p. 455–471. In: Fundamentals of Aquatic Toxicology. M. G. Rand and S. R. Petrochelli (eds.). Hemisphere Publishing Corporation, Washington, D.C.

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber- Sanwall E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker B., Walker M, Wall DH. 2000. Global biodiversity scenarios for the year 2100. Science, 287: 1770-1774.

Scott GR, Sloman KA, 2004. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquat. Toxicol., 68: 369-392.

Semlitsch RD. 1993. Asymmetric competition in mixed populations of tadpoles of the hybridogenetic Rana esculenta complex. Evolution, 47: 510-519.

Shinn C, Marco A, Serrano L. 2008. Inter- and intra-specific variation on sensitivity of larval amphibians to nitrite. Chemosphere, 7: 507-514.

Sievers M, Hale R, Swearer SE, Parris KM. 2018. Contaminant mixtures interact to impair predator-avoidance behaviours and survival in a larval amphibian. Ecotoxicol. Environ. Saf., 161: 482-488.

Simplício N, Muniz D, Rocha F, Martins D, Dias Z, Farias B, Oliveira-Filho E. 2017. Comparative analysis between ecotoxicity of nitrogen-, phosphorus-, and potassium-based fertilizers and their active ingredients. Toxic., 5: 2.

Şişman T, Keskin MÇ, Dane H, Adil Ş, Geyikoğlu F, Çolak S, Canpolat E. 2021. Marsh Frog (*Pelophylax ridibundus*) as a Bioindicator to Assess Pollution in an Agricultural Area. Pakistan J. Zool.,53(1): 337-349.

Slaby S, Marin M, Marchand G, Lemiere S. 2019. Exposures to chemical contaminants: what can we learn from reproduction and development endpoints in the amphibian ecotoxicology literature? Environ. Pollut., 248: 478-495.

Smith DC. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology, 68: 344-350.

Smith GR. 2007. Lack of effect of nitrate, nitrite, and phosphate on wood frog (Rana sylvatica) tadpoles. Appl. Herpetol., 4: 287-291.

Sodhi NS, Bickford D, Diesmos AC, Lee TM, Koh LP, Brook BW, Sekerciogl, C H, Bradshaw CJA. 2008. Measuring the meltdown: drivers of global amphibian extinction and decline. PloS One 3, e1636.

Soucek DJ, Dickinson A. 2016. Influence of chloride on the chronic toxicity of sodium nitrate to Ceriodaphnia dubia and Hyalella azteca. Ecotoxicolog 25: 1406-1416.

Teplitsky C, Plenet S, L´ena, JP, Mermet N, Malet E, Joly P. 2005. Escape behaviour and ultimate causes of specific induced defences in an anuran tadpole. J. Evol. Biol., 18: 180-190.

Todd BD, Bergeron CM, Hepner MJ, Hopkins WA. 2011. Aquatic and terrestrial stressors in amphibians: A test of the double jeopardy hypothesis based on maternally and trophically derived contaminants. Environ. Toxicol. Chem., 30: 2277–2284.

Trudeau VL, Thomson P, Zhang WS, Reynaud S, Navarro-Martín L, Langlois VS. 2020. Agrochemicals disrupt multiple endocrine axes in amphibians. Mol. Cell. Endocrinol. 513, 110861.

Van Meter RJ, Adelizzi R, Glinski DA, Henderson WM. 2019. Agrochemical mixtures and amphibians: the combined effects of pesticides and fertilizer on stress, acetylcholinesterase activity, and bioaccumulation in a terrestrial environment. Environ. Toxicol. Chem., 38: 1052-1061.

Waldner F, De Abelleyra D, Ver´on SR, Zhang M, Wu B, Plotnikov D, Bartalev S, Lavreniuk M, Skakun S, Kussul N, Le Maire G, Dupuy S, Jarvis I, Defourny P. 2016. Towards a set of agrosystem-specific cropland mapping methods to address the global cropland diversity. Int. J. Rem. Sens. 37: 3196-3231.

Wang M, Chai L, Zhao H, Wu, M, Wang H. 2015. Effects of nitrate on metamorphosis, thyroid and iodothyronine deiodinases expression in Bufo gargarizans larvae. Chemosphere, 139: 402-409.

Warne RW, Crespi EJ, Brunner JL. 2011. Escape from the pond: stress and developmental responses to ranavirus infection in wood frog tadpoles. Funct. Ecol., 25: 139-146.

Watkins TB. 1996. Predator-mediated selection on burst swimming speed performance in tadpoles of the Pacific tree frog, Pseudacris regilla. Physiol. Zool., 69: 154-167.

Werner EE, Anholt BR. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. American Naturalist, 142: 242-272.

Whitfield SM, Alvarado G, Abarca J, Zumbado H, Zuñiga I, Wainwright M, Kerby J.2017. Differential patterns of Batrachochytrium dendrobatidis infection in relict amphibian populations following severe disease-associated declines. Dis. Aquat. Org., 126: 33-41

Wilbur HM. 1997. Experimental ecology of food webs: complex systems in temporary ponds. Ecology, 78: 2279-2302.

Won HS, Kang SJ, Lee BJ. 2009.Action mechanism and structural requirements of the antimicrobial peptides, gaegurins, Biochimica et Biophysica Acta (BBA) - Biomembranes, 1788(8): 1620-1629.

Wünschmann S, Wünschmann Wüst-Ackermann P, Randler C, Vollmer C. 2017. Learning Achievement and Motivation in an Out-of-School Setting—Visiting Amphibians and Reptiles in a Zoo Is More Effective than a Lesson at School Res Sci Educ, 47: 497.

Yüksek T, Yüksek F, Sütlü E. 2013. Rize Yöresinde Çay Tarımında Gübreleme Sorunları ve Sürdürülebilir Çay Tarımı İçin Yeni Straterjiler. II. Rize Kalkınma Sempozyumu, Rize, 3-4 Mayıs 2013, Bildiri Özetleri Kitabı, 53-54 .

Zambrano-Fern´andez S., Zamora-Camacho FJ, Arag´on, P. 2020. Does chronic exposure to ammonium during the pre-metamorphic stages promote hindlimb abnormality in anuran metamorphs? A comparison between natural-habitat and agrosystem frogs. Acta Herpetol., 15: 119-123.

Zambrano-Fernández, S, Zamora-Camacho F.J, Aragón, P 2022. Direct and indirect effects of chronic exposure to ammonium on anuran larvae survivorship, morphology, and swimming speed. Chemosphere, 287: 132349.

Zhelev Z, Popgeorgiev GS, Mehterov NH. 2014. Changes in the basic morphophysiological parameters in the populations of Pelophylax ridibundus (Amphibia: Ranidae) from anthropogenically polluted biotopes in Southern Bulgaria. Part 1 Bulg. J. Agric. Sci., 20(5): 1202-1210.

Zhelev Z, Popgeorgiev GS, Mehterov NH. 2015. Changes in the hepatosomatic index and condition factor in the populations of Pelophylax ridibundus (Amphibia: Ranidae) from anthropogenically polluted biotopes in Southern Bulgaria. Pt II Bulg. J. Agric. Sci., 21(3): 534-539.